Doctoral thesis/Tesis Doctoral

Influencia del consumo de antiinflamatorios no esteroideos en la incidencia de cáncer de mama

Influence of non-steroidal antiinflammatory drugs use on breast cancer incidence

Author: María de Pedro Cárdenas

Directors:

Marina Pollán

Javier Llorca

Universidad de Cantabria

Facultad de Medicina, Departamento de Ciencias Médicas y Quirúrgicas

Santander, 2015

Marina Pollán Santamaría, investigadora del Centro Nacional de Epidemiología, Instituto de Salud Carlos III, y Javier Llorca Díaz, Catedrático de Medicina Preventiva y Salud Pública de la Universidad de Cantabria,

CERTIFICAN:

Que el trabajo titulado ***Influence of non-steroidal antiinflammatory drugs use on breast cancer incidence*** que presenta Dña. ***María de Pedro Cárdenas*** para optar al grado de Doctor ha sido realizado bajo nuestra dirección y reúne las características de originalidad y rigor científico requeridas.

Y para que conste y surta los efectos oportunos, expiden el presente certificado en Santander, a 15 de junio de dos mil quince.

ACKNOWLEDGMENTS

First and foremost, I would like to express my special appreciation and thanks to my director Professor Dr. Javier Llorca, for his encouragement, his help and his proverbial patience during all these years. I would also like to thank my director Dr. Marina Pollán for her generous and irreplaceable collaboration.

I would especially like to thank Dr. Christine Teal, my mentor during my clinical rotation at George Washington University Hospital, for her valuable lessons and her warm hospitality. I would also like to thank my colleagues at Marqués de Valdecilla and Nuevo Belén University Hospitals, for their friendship and support.

Finally, I would like to thank my family and my husband for their understanding and, of course, their love.

TABLE OF CONTENTS

RESUMEN (EN ESPAÑOL)

GENERALIDADES Y CLASIFICACIÓN DE LOS TUMORES DE MAMA

El cáncer de mama es la neoplasia más frecuentemente diagnosticada en el mundo: afecta a una de cada ocho mujeres en España y una de cada seis en Estados Unidos (Chen, 2014). Supone la primera causa de muerte por cáncer en mujeres y la primera causa de muerte en mujeres de 40 a 59 años (Chen, 2014). La tasa de mortalidad por cáncer de mama en España es de 17.5 por 100000 habitantes/año, que se traduce en alrededor de 6000 muertes anuales (Centro Nacional de Epidemiología, 2013). Sin embargo, presenta una de las mayores tasas de supervivencia a los 5 años (82% en España y 89.2% en Estados Unidos) (Howlander *et al.,* 2014; Ferlay *et al.,* 2015). Se trata, por lo tanto, de un problema epidemiológico de primer orden, que justifica por sí mismo los recursos puestos a su disposición desde hace décadas.

A lo largo de los años, se han venido utilizando diversas características de los tumores de mama para conseguir una clasificación útil en cuanto al pronóstico y al tratamiento de la enfermedad. Inicialmente se emplearon criterios clínicos, tales como la edad de la paciente, el tamaño del tumor, la afectación ganglionar y la presencia de metástasis; más tarde, se añadieron criterios anatomopatológicos, como el grado histológico y las características inmunohistoquímicas -fundamentalmente, la expresión de receptores para estrógenos, progesterona y factor de crecimiento epidérmico humano 2 [HER2]. Toda esta información se utiliza para valorar el pronóstico de la enfermedad y establecer la mejor actitud terapéutica. Así, la expresión de receptores estrogénicos en un tumor (definida a menudo como "receptor de estrógeno positivo" o "RE+") sugiere que los estrógenos están implicados en su crecimiento y que, por lo tanto, es candidato a un tratamiento antiestrogénico. Por el mismo motivo, los tumores que no expresan dichos receptores dependen de otros factores distintos a los estrógenos, no estando indicado el tratamiento hormonal. Dos de cada tres cánceres de mama expresan algún tipo de receptor hormonal.

A pesar de dichos modelos, no se ha encontrado hasta ahora ningún sistema de clasificación de los tumores de mama que explique la alta variabilidad en la evolución y en la respuesta ante un mismo tratamiento. Por este motivo se investigan constantemente nuevos criterios que puedan proporcionen información de forma más precisa. Un ejemplo de estas nuevas clasificaciones es la basada en análisis de expresión genética, que define cinco subtipos intrínsecos de cáncer de mama (Luminal A, Luminal B, HER2, basal y bajo en claudina) y un

subtipo de tejido mamario normal (Perou *et al.,* 2000; Sørlie *et al.,* 2001; Herschkowitz *et al.,* 2007; Prat *et al.,* 2010) (Tabla 1). Entre estos grupos se han encontrado diferencias cruciales respecto a la incidencia, supervivencia y respuesta al tratamiento, completando la información procedente de los criterios tradicionales de clasificación (Prat *et al.,* 2011; Goldhirsch *et al.,* 2013) (Tabla 2).

FACTORES DE RIESGO PARA EL CÁNCER DE MAMA

De modo similar a otras enfermedades, el cáncer de mama está sujeto a factores de riesgo tanto ambientales como genéticos para su desarrollo. La mayoría de las mujeres diagnosticadas (80-85%) presentan cánceres de mama esporádicos, es decir, sin historia familiar relevante ni predisposición genética. Estas mujeres, debido a diversos factores de riesgo ambientales, adquieren múltiples mutaciones, que se acumularán a lo largo de su vida, antes de la aparición del tumor. Sin embargo, existe un grupo de mujeres más pequeño (15-20%), que corresponde al cáncer de mama hereditario. Estas pacientes suelen pertenecer a familias con múltiples casos de cáncer de mama, y ya presentan alguna de esas mutaciones en el momento de su nacimiento, precisando, por lo tanto, menos tiempo para acumular las mutaciones restantes, de modo que el tumor se desarrolla frecuentemente a una edad más temprana (Isaacs *et al.,* 2012).

Los factores de riesgo más importantes para el desarrollo del cáncer de mama son el sexo femenino y la edad, pudiendo considerarse a los tumores pre- y postmenopáusicos como entidades distintas, con arreglo a sus características hormonales, moleculares e histológicas. La incidencia de cáncer de mama aumenta rápidamente durante la vida fértil y tras la menopausia, después de lo cual continúa incrementándose, aunque a un ritmo menor [Cancer Incidence in Five Continents, Vol. X (electronic version)].

Entre los factores de riesgo ambientales más relevantes para el desarrollo del cáncer de mama se encuentran los siguientes: la edad de la menarquia, la edad del primer parto y el número de biopsias de mama. También han demostrado su influencia en el riesgo de cáncer de mama la edad de la menopausia, la paridad, la lactancia materna, los abortos espontáneos o inducidos y los niveles de hormonas, tanto de origen endógeno como exógeno. Por último, existe una larga lista de factores con mucha menos relevancia, fundamentalmente relacionados con la

alimentación y el estilo de vida, que presentan un impacto muy débil en el desarrollo de la enfermedad y que, en muchos casos, son indicadores indirectos de los factores hormonales y reproductivos. Concretamente, la exposición a diferentes sustancias y medicamentos -salvo la terapia hormonal sustitutiva- ha sido ampliamente estudiada, pero con resultados dispares.

INFLAMACIÓN Y CÁNCER DE MAMA

Aunque, como se ha mencionado anteriormente, los factores de riesgo más importantes para el cáncer de mama sean hormonales y reproductivos, se ha demostrado el papel de la inflamación en la carcinogénesis mamaria, concretamente a través de la vía metabólica de la ciclooxigenasa (COX) y las prostaglandinas (PG).

Se ha demostrado, mediante estudios moleculares, que la sobreexpresión de la isoforma inducible de la ciclooxigenasa (COX-2) es una pieza clave en todas las fases del desarrollo tumoral, detectándose tanto en lesiones premalignas (displasia y atipia), como en el carcinoma *in situ*, invasivo, y, particularmente, en al enfermedad metastásica. De hecho, algunos estudios han sugerido una interesante asociación entre la expresión de *COX-2* y la aparición de criterios de agresividad de la enfermedad: mayor tamaño tumoral, bajo grado de diferenciación, alta tasa de proliferación, formación de metástasis, ausencia de receptores hormonales y sobreexpresión de *HER2* (Ristimäki *et al.,* 2002; Subbaramaiah *et al.,* 2002; Denkert *et al*., 2003; Shim *et al*., 2003; Wulfing *et al.,* 2003; Boland *et al.,* 2004; Tan *et al*., 2004; Perrone *et al*., 2005; Takeshita *et al*., 2005; Barnes *et al*., 2006). De modo análogo, otros estudios han propuesto la vía COX/PG como una posible diana para prevenir la progresión del carcinoma *in situ* hacia la enfermedad invasiva (Boland *et al.,* 2004; Half *et al.,* 2002; Soslow *et al.,* 2000; Watanabe *et al.,* 2003; Shim *et al.,* 2003; Tan *et al.,* 2004). Por otro lado, la expresión de *COX-2* en tejido mamario sano es prácticamente inexistente (Wu, 1996; Dubois *et al*., 1998), salvo en áreas focales en las que se detectan otros cambios moleculares, como el silenciamiento de CDKN2A *CDKN2A* ($p16^{INK4a}$), lo cual podría significar que la sobreexpresión de COX-2 dentro de la tumorogénesis mamaria es un acontecimiento muy precoz (Crawford *et al.,* 2004). Otro matiz interesante es que, a diferencia de lo que ocurre en modelos de cáncer colorrectal, en los que se ha identificado la sobreexpresión de *COX-2* en tejdio estromal (Oshima *et al.,* 1996), la activación de *COX-2* en el caso del cáncer de mama se ha detectado exclusivamente en células epiteliales (Hamid *et al.,* 1999; Howe *et al.,* 2001; Howe *et al.,* 2002; Nakatsugi *et al.,* 2000; Robertson *et al.,* 1998).

Por otro lado, la prostaglandina E_2 (PGE_2), producto principal de *COX-2*, también se detecta en mayores concentraciones en tejido mamario neoplásico (Bennett *et al.,* 1983). La asociación entre niveles altos de PGE_2 y la aparición del cáncer de mama parece obedecer a un incremento de la actividad de la aromatasa, que, a su vez, conduce a un aumento de la síntesis de estrógenos en el epitelio y el estroma mamarios (Brueggemeier *et al.,* 2005). Dicho aumento de los niveles de prostaglandinas contribuye a la carcinogénesis mediante diversos mecanismos: aumento de la mitosis, mutagénesis y angiogénesis, formación de metástasis, inhibición de la apoptosis e inmunosupresión.

Desde el punto de vista experimental, se ha demostrado en numerosos estudios con ratones durante los últimos treinta años que la inhibición farmacológica de la COX (tanto con AINEs tradicionales como con inhibidores selectivos de COX-2) tiene un efecto supresor sobre el cáncer de mama, lo cual sugiere su posible uso quimiopreventivo. Por otro lado, el bloqueo genético de *COX-2* ha demostrado disminuir la formación de tumores (Howe *et al.,* 2001; Howe, 2005). A la inversa, la sobreexpresión transgénica de *COX-2* basta para inducir la formación de tumores en hembras multíparas, lo cual supone una prueba directa del potencial ongonénico de *COX-2 in vitro* (Liu *et al.,* 2001).

Respecto a la expresión de receptores hormonales, existe evidencia sobre la eficacia de los inhibidores selectivos de *COX-2* en la disminución de los tumores negativos para receptores de estrógenos (Boland *et al.,* 2004; Denkert *et al.,* 2003; Wulfing *et al.,* 2003, Ristimäki *et al.,* 2002). Varios estudios con ratones transgénicos para *HER2* han descrito una menor formación de tumores RE negativos tras la administración de Celecoxib (Howe *et al.,* 2002; Lanza-Jacobi *et al.,* 2003), de manera que la inhibición de esta vía podría ser de utilidad no sólo para los tumores que expresan *HER2*, sino también para los RE negativos.

Se han propuesto otros puntos de la vía metabólica de los eicosanoides, como el bloqueo de la PGE_2 sintetasa, el bloqueo de los receptores de prostaglandinas (Howe *et al.,* 2002; Chang *et al.,* 2004; Cheng *et al.,* 2006) y, más recientemente, la inactivación epigenética de PGE_2 (Blacklund *et al.,* 2005; Ding *et al.,* 2005; Myung *et al.,* 2006; Wolf *et al.,* 2006; Yan *et al.,* 2004; Mann *et al.,* 2006).

DATOS EPIDEMIOLÓGICOS

A pesar su consistencia y abundancia, los datos experimentales no se han visto corroborados con la misma rotundidad desde el punto de vista epidemiológico. Durante los últimos 35 años, se han publicado numerosos estudios, con resultados irregulares y, en muchos casos, no significativos. A pesar de ello, la evidencia demuestra un modesto efecto protector de los antiinflamatorios no esteroideos (AINEs) frente al cáncer de mama. Es llamativa la práctica ausencia de ensayos clínicos aleatorizados y el hecho de que los estudios observacionales se basan fundamentalmente en datos de consumo proporcionados por los propios pacientes. Dentro de los estudios observacionales, los estudios de casos y controles tienden a presentar un efecto mayor que los estudios de cohortes, probablemente a causa del mayor número de sesgos al que se someten, como el de recuerdo o el de selección. Este matiz es especialmente relevante en el caso de los datos de inhibidores selectivos de COX-2 e ibuprofeno, que proceden fundamentalmente de estudios de casos y controles, de manera que la magnitud de su efecto podría ser menor. Por otro lado, la mayoría de los estudios de cohortes no actualizan la información que proporcionan sus participantes al comienzo, y, en algunos casos, esa información se obtiene de recetas médicas, que no reflejan de manera fiel el consumo real, de modo que los datos sobre consumo son, en muchos casos, muy anteriores a la aparición del tumor. Por otro lado, muchos de estos medicamentos se venden sin prescripción, lo cual dificulta aún más la cuantificación del consumo.

Otra posible explicación para la disparidad de los resultados consistiría en que la inhibición de COX-2 no es igual de intensa con todos los antiinflamatorios, observándose mayores reducciones del riesgo con unos que con otros. Finalmente, parte de esa heterogeneidad podría explicarse por el efecto diferencial de los AINEs en función del genotipo de COX-2, del patrón de expresión hormonal y de la presencia de enfermedades inflamatorias.

METAANÁLISIS Y REVISIÓN SISTEMÁTICA

Con el ánimo de responder al menos a alguna de estas incógnitas, se ha realizado un metaanálisis y revisión sistemática, actualizada hasta el 24 de octubre de 2013. Los metaanálisis se realizaron de manera separada para estudios de casos y controles y estudios de cohortes, para diferentes tipos de AINEs y para expresión de diferentes receptores

hormonales. Con este análisis no sólo se pretende asignar un RR/OR a cada exposición, sino también generar hipótesis sobre el desarrollo del cáncer de mama e intentar explicar las inconsistencias detectadas en estudios anteriores.

Nuestros resultados confirmaron una disminución del 20% en la incidencia de cáncer de mama invasivo con el consumo de AINEs de manera global, con reducciones similares para aspirina, paracetamol e inhibidores selectivos de COX-2, y una disminución más discreta para ibuprofeno (OR 0.87). Aunque estos datos no difieren de los presentados en metaanálisis previos, nuestro estudio incluye dos novedades: el efecto protector de los inhibidores selectivos de COX-2 (OR 0.90) y el efecto protector de la aspirina frente a los tumores que expresan receptores de estrógenos y progesterona (OR 0.73 en ambos casos).

La información sobre el efecto de los inhibidores selectivos de COX-2 es escasa (Rahme *et al.*, 2005; Harris *et al.,* 2006; Cronin-Fenton *et al.*, 2010; Ashok *et al.*, 2011; Vinogradova *et al.,* 2011), fundamentalmente debido a la suspensión de la mayoría de los estudios tras observar el aumento del riesgo tromboembólico de dichos fármacos. Sin embargo, su efecto protector sobre el cáncer de mama parece más intenso que el de los AINEs tradicionales, y existen revisiones recientes que defienden su seguridad, dentro de un cierto rango de dosis (Coogan *et al.*, 1999). Se necesitan más estudios para confirmar dicha reducción, sobre todo en relación con su efecto diferencial en tumores con receptores hormonales positivos y negativos.

También son escasos los estudios publicados que contienen información sobre los distintos tipos moleculares y patrones hormonales de los tumores mamarios (Terry *et al.*, 2004; Zhang *et al.*, 2005; Kirsh *et al.*, 2007; Brasky *et al.*, 2011; Marshall *et al.*, 2005; Gallicchio *et al.*, 2007; Friis *et al.*, 2008; Eliassen *et al.*, 2009; Bardia *et al.*, 2011; Zhang *et al.,* 2012; Zhang *et al.*, 2008). Su inclusión en los metaanálisis previos ha sido en algunos casos sólo parcial, debido a que no se habían publicado aún (Zhang *et al.*, 2005; Kirsh *et al.*, 2007; Brasky *et al.*, 2011,; Gallicchio *et al.,* 2007; Gill *et al.,* 2007; Friis *et al.*, 2008; Eliassen *et al.*, 200; Bardia *et al.,* 2011; Zhang *et al.*, 2012; Zhang *et al.*, 2008) o a que los datos no eran suficientes para el metaanálisis (Jonsson *et al.*, 2013; Eliassen *et al.,* 2009). Sólo hay dos metaanálisis recientes que incluyen esta información (Luo *et al.,* 2012; Tolentino *et al.*, 2012), pero sólo se evaluó el efecto de la aspirina, sin valorar los demás AINEs.

En el momento de nuestra revisión y metaanálisis, se encontraron 12 publicaciones que evaluaban el efecto de los AINEs en tumores con receptores hormonales positivos, lo cual ha

permitido un análisis separado de estos casos, observándose un mayor descenso del riesgo de cáncer de mama con RE positivos que del cáncer de mama en general.

Aparte de la heterogeneidad de los resultados que ha sido mencionada previamente, hay que tener en cuenta varias limitaciones que han podido afectar al resultado del metaanálisis. En primer lugar, existe una importante heterogeneidad de dosis y duraciones de tratamiento, que ha resultado muy difícil unificar, debido a que esa información quedaba definida de manera particular por cada estudio. Aunque algunos metaanálisis previos han evaluado la relación dosis-respuesta, hemos considerado que la falta de homogeneidad a la hora de determinar las dosis resta fiabilidad a este tipo de análisis. En segundo lugar, varias publicaciones utilizan el epígrafe "cualquier AINE" sin aclarar qué fármacos se incluyen. A pesar de ello, en nuestro metaanálisis se han combinado esos resultados, siendo conscientes de la posible heterogeneidad de ese grupo. En tercer lugar, existen varias características moleculares que no se han reflejado de manera homogénea en los resultados: por ejemplo, la expresión de HER2, que parece relevante en cuanto al mecanismo protector de los AINEs en el cáncer de mama, aparece en muy pocas publicaciones, lo cual ha impedido incluir este punto en el metaanálisis. De manera análoga, la positividad para receptores de estrógenos y de progesterona se expresa como RE/RP, sin especificar si se trata de un receptor o de los dos, mientras que otros estudios sí los consideran por separado. Es llamativa la falta de datos sobre receptores hormonales en los estudios de cohortes -sólo 7 incluyen esta información (Gill *et al.,* 2007; Friis *et al.,* 2008; Ready *et al.,* 2008, Gierarch *et al.,* 2008; Eliassen *et al.,* 2009; Bardia *et al.,* 2011; Zhang *et al.,* 2012). Este hecho podría explicar, al menos en parte, la menor reducción del riesgo observada en estos estudios.

Para concluir, este metaanálisis podría conducir al estudio de varias hipótesis interesantes como la posibilidad de que los distintos genotipos de COX-2 o la presencia de enfermedad inflamatoria puedea modificar el efecto de los AINEs sobre la incidencia de la enfermedad, o el efecto específico de los AINEs en cada subtipo molecular de cáncer de mama.

INTRODUCTION

GENERAL ASPECTS AND DEFINITIONS OF BREAST CANCER

Breast cancer is the most frequently diagnosed neoplasia globally. It represents the first cause of death from cancer in women and the first cause of death overall in women between 40 and 59 years of age (Chen, 2014). One in six women in the United States and one in eight in Spain will develop breast cancer during their lifetime (Chen, 2014). The mortality rate of breast cancer in Spain in 2013 was 17.5 deaths per 100,000 population/year, which results in about 6000 deaths annually (Centro Nacional de Epidemiología, 2013).

Although breast cancer risk factors are extensively discussed below, it is important to bear in mind that sex and age are the most determinant. Breast cancer incidence increases rapidly during the years of hormonal activity and after menopause and stabilizes at 70 years of age [Cancer Incidence in Five Continents, Vol. X (electronic version)].

No classification system of breast tumors has so far been able to explain the high variability in evolution and response given the same treatment. This is the reason why new criteria are constantly under research in order to obtain a more useful and more accurate outcome prediction. Studies based on global gene expression analyses (Perou *et al.,* 2000; Sørlie *et al.,* 2001; Van't Veer *et al.,* 2002; Naderi *et al.,* 2007) have started to yield further understanding to this subject. During the last 15 years, five molecular 'intrinsic' subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Claudin-low) and a Normal Breast-like group have been identified and thoroughly studied (Table 1) (Perou *et al.,* 2000; Sørlie *et al.,* 2001; Herschkowitz *et al.,* 2007; Prat *et al.,* 2010). These groups of tumors have been found to differ in crucial features such as incidence, survival and response to treatment, which in turn has expanded the knowledge provided by traditional classification criteria (Prat *et al.,* 2011). A surrogate clinical subtyping based on immunohistochemical features was reached at the 2013 St. Gallen International Breast Cancer Conference (Goldhirsch *et al.,* 2013) (Table 2).

Table 1. Molecular subtypes of breast cancer and their main features

Molecular subtype	Frequency	ER;PR; HER2	CK5/6 EGFR	Proliferation	Genetic features	Grade	p53 mutations	Prognosis
Basal-like	8-20%	ER-;PR-;HER2-	+	High	KRT5,CDH3,ID4,FABP7, KRT17,TRIM29,LAMC2	High	++	Poor
HER2-enriched	10-15%	ER-;PR-;HER2+	+/-	High	ERBB2,GRB7	High	++	Poor
Normal breast-like	6-10%	HER2+	+	Low	PTN,CD36,FABP4, AQP7,ITGA7	Low	-	Intermediate
Luminal A	55-60%	ER+;PR+;HER2-	-	Low	ESR1,GATA3,KRT8,KRT18,XBP1, FOXA1,TFF3,CCND1,LIV1	Low	-	Excellent
Luminal B	10-15%	ER+/-;PR+/- HER2-/+	-	High	ESR1,GATA3,KRT8,KRT18,XBP1, FOXA1,TFF3,SQLE,LAPTM4B	Intermediate	+/++	Intermediate/poor
Claudin-low	5%	ER-;PR-;HER2-	+/-	High	CD44,SNAI3	High	++	Poor

Modified from Yersal et al., 2014

Table 2. Intrinsic subtypes of breast cancer

Intrinsic subtype	Clinico-pathologic surrogate definition	
Luminal A	"Luminal A-like" all of ER+ and PR+ HER2- Ki-67 low Low risk of recurrence based on multi-gene-expression assay (if available)	
Luminal B	"Luminal B-like (HER2 -)" ER+ HER2- and at least one of: Ki-67 high PR low or negative High risk of recurrence based on multi-gene-expression assay (if available)	"Luminal B-like (HER2+) ER+ HER2 over-expressed or amplified Any Ki67 Any PR
ErbB2 over-expression	"HER2 + (non-luminal)" HER2 over-expressed or amplified ER and PR absent	
Basal-like	"Triple negative (ductal)" ER and PR absent HER2-	

From Goldhirsch et al., 2013

RISK FACTORS FOR BREAST CANCER

Any cancer is determined to some extent by factors that alter the cell cycle regulation, and breast cancer is not an exception. Most of these factors are environmental, meaning that individuals acquire them during their lifetime. When a critical number of factors accumulate, disease develops and appears. This explains, on the one hand, the direct link between breast cancer incidence and age and, on the other, the epidemiological, histological and molecular differences found amongst different populations. A less relevant group, at least quantitatively, consists of hereditary factors or germ-line gene mutations, which predispose their carrier to develop cancer from birth.

Among the environmental factors, the most relevant for developing breast cancer are related to the hormonal activity and, therefore, to the reproductive history: age at menarche, parity and age at first birth, age at menopause, breastfeeding, miscarriage and induced abortion, and hormone levels (either endogenous or exogenous).

In 1989, Gail *et al.* published a predictive model to assess the individual risk of breast cancer, considering the contribution of each of both genes and reproductive factors. Their model provides a risk estimate for breast cancer at a determined age during a determined period of time based on four criteria: age at menarche, age at first birth, number of breast biopsies and number of first-degree relatives with breast cancer (Tables 3 and 4). The relative importance of genes and environment in this model is consistent with the aforementioned proportions (80-85% sporadic and 15-20% familiar cancers). Despite its limitations, such as the exclusion of other confirmed risk factors, Gail's proposal has been the most widely used by posterior studies (Costantino *et al.,* 1999; Rockhill *et al.,* 2001), even if it requires recalibration depending on the studied population, as observed in Spain (Pastor-Barriuso *et al*., 2013). The only relevant modification consisted on adding mammographic density (Chen *et al.,* 2006).

Table 3. Risk estimate for breast cancer after 10, 20 and 30 years of follow-up (%) for Caucasian women in the US

Age at start of follow up	Years of follow up	RR after follow up*	Initial RR*♦					
			1.0	2.0	5.0	10.0	20.0	30.0
20	10		0.0	0.1	0.2	0.5	1.0	1.4
	20		0.5	1.0	2.5	4.9	9.5	14.0
	30		1.7	3.4	8.3	15.9	29.3	40.5
30	10		0.5	0.9	2.3	4.4	8.7	12.8
	20		1.7	3.3	8.1	15.6	28.8	39.9
	30	1.0	3.2	4.8	9.5	16.9	29.9	40.8
		2.0	4.7	6.3	10.9	18.2	30.9	41.7
		5.0	8.9	10.4	14.9	21.8	34.0	44.3
		10.0	15.6	17.1	21.2	27.6	38.8	48.3
		20.0	27.6	28.8	32.3	37.8	47.4	55.5
		30.0	37.7	38.7	41.8	46.4	54.7	61.7
40	10		1.2	2.5	6.1	11.8	22.2	31.3
	20	1.0	2.8	4.0	7.5	13.1	23.4	32.4
		2.0	4.3	5.5	8.9	14.5	24.5	33.4
		5.0	8.6	9.7	13.1	18.3	28.0	36.4
		10.0	15.4	16.4	19.5	24.4	33.3	41.1
		20.0	27.4	28.4	30.9	35.2	42.7	49.5
		30.0	37.7	38.5	40.7	44.3	50.8	56.6
	30	1.0	4.4	5.6	9.1	14.6	24.6	33.5
		2.0	7.4	8.6	11.9	17.3	27.0	35.6
		5.0	15.9	17.0	20.0	24.9	33.7	41.5
		10.0	28.3	29.2	31.8	35.9	43.4	50.0
		20.0	47.5	48.1	50.0	53.1	58.5	63.4
		30.0	61.2	61.6	63.1	65.3	69.3	72.8
50	10		1.6	3.1	7.6	14.6	27.1	37.7
	20		3.2	6.4	15.1	27.9	47.8	61.9
	30		4.4	8.5	19.9	35.5	57.8	71.7
60	10		1.8	3.6	8.6	16.5	30.1	41.5
	20		3.0	5.9	14.0	25.9	44.6	58.2
70	10		1.4	2.7	6.7	12.9	24.1	33.7

**The initial RR corresponds with the age at start of follow up. If the initial age is <50 and if the initial age plus the years of follow up are >50, a RR after 50 years should be specified.If the initial age is ≥50, only the initial relative risk is necessary. If the initial age is <50 and the initial age plus the years of follow up is ≤ 50, only the initial relative risk is necessary. ♦ Values in columns are probability projections expressed in percentage.*

From Gail et al., 1989

Table 4. Relative risk of breast cancer based on Gail model.

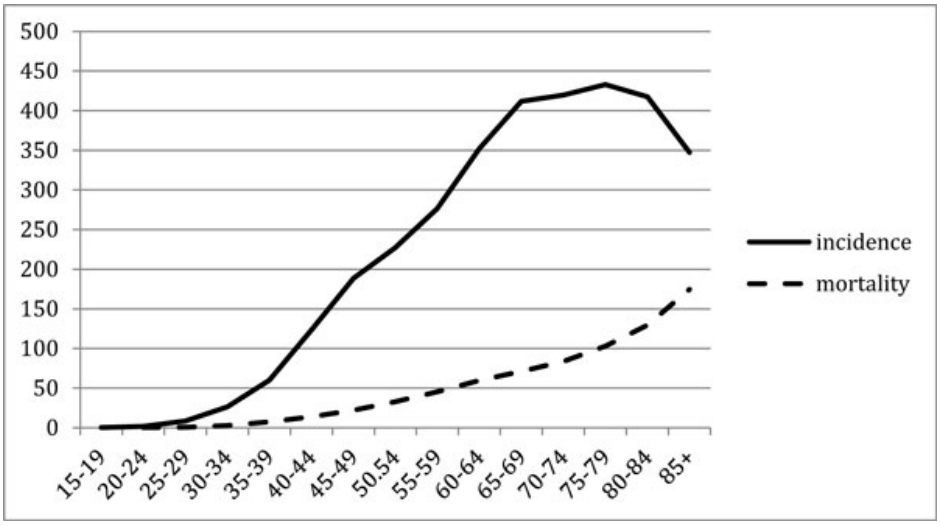
Risk factor		Relative risk
Category A: age at menarche		
>14 years		1.00
12-13 years		1.10
<12 years		1.21
Category B: number of breast biopsies and age		
0	Any age	1.00
1	<50 years	1.70
	≥50 years	1.27
2	<50 years	2.88
	≥50 years	1.62
Category C: number of first-degree relatives with breast cancer and age at first birth		
0	<20 years	1.00
	20-24 years	1.24
	25-29 years or nulliparous	1.55
	≥30 years	1.93
1	<20 years	2.61
	20-24 years	2.68
	25-29 years	2.76
	≥30 years	2.83
≥2	<20 years	6.80
	20-24 years	5.78
	25-29 years or nulliparous	4.91
	≥30 years	4.17

Modified from Armstrong et al., 2000

ENVIRONMENTAL FACTORS

Although the aim of this thesis is to review in depth the role of antiinflammatory drugs, the influence of other environmental factors has to be also considered. Not only they do account for 80 to 85% of breast cancer cases, but some of them -particularly hormonal and reproductive factors- have largely contributed to a better understanding of breast cancer pathogenesis and have set the basis for further genetic and molecular classifications.

Figure 1. Age-adjusted breast cancer risk incidence and mortality rates/100,000, 2005-2009 in the United States (SEER Cancer Statistics Review)

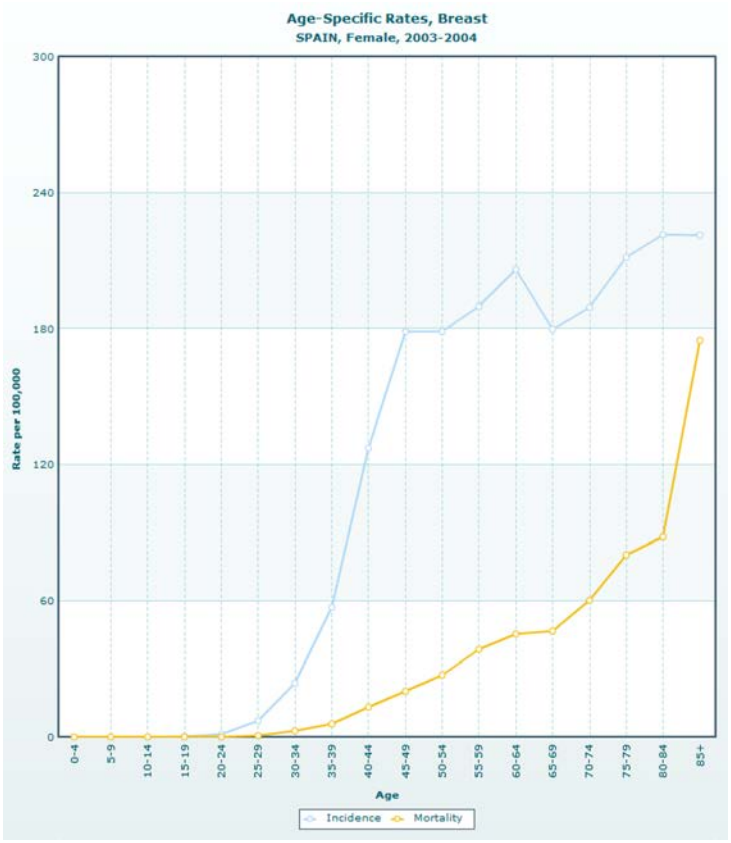


From Cappellani et al., 2013

AGE AND SEX

The main risk factors for breast cancer are being female and age. Breast cancer is 100-fold more frequent in women than in men, and its incidence increases rapidly until 70 years of age, at which point this increase is less steep (Howlader *et al.,* 2009) (Figures 1-2).

Figure 2. Age-adjusted breast cancer incidence and mortality rates/100,000 2003-2004 in Spain



From Ferlay et al., 2013

BENIGN BREAST DISEASE

Most cases of benign breast disease do not pose a major risk of cancer, except multiple lesions, in which case a slightly higher risk can be observed (10 year-RR =1.8). Proliferative lesions, however, do increase breast cancer risk (RR 1.3-2), especially if they present focal cytological atypia (RR 4-6), if they are lobular rather than ductal and even more if they are multifocal (RR ≥10) (Chen, 2014) (Table 5). More recently, a more accurate association between benign breast disease and breast cancer risk has been observed, based on mammographic breast density: women with atypical hyperplasia and high density breasts present a 5-fold higher risk of breast cancer (Table 6) (Tice *et al.,* 2013). Breast density is discussed later in the text.

Table 5. Benign breast lesions, histological findings and relative risk of breast cancer

RR	Proliferation	Histological findings
≤1.5	Minimal	Fibrocystic breast Benign tumor Trauma Infection Apocrine and squamous metaplasia Chronic disease (diabetes, sarcoidosis)
1.5-2	No atypia	Ductal hyperplasia Complicated fibroadenoma Papilomatosis Radial scar Ductal adenosis
≥2	Atypia	Atypical lobular hyperplasia Atypical ductal hyperplasia

From Santen et al, 2005

Table 6. Breast cancer risk associated with benign breast disease cross-classified with breast density

Benign breast disease	BIRADS breast density, HR (95% CI)*			
	1	2	3	4
Nonproliferative	0.85 (0.56-1.28)	1.0 (reference)	1.51 (1.28-1.78)	2.15 (1.73-2.68)
	P = .44		P < .001	P < .001
Proliferative without atypia	0.67 (0.30-1.52),	1.37 (1.11- 1.69),	2.02 (1.68-2.44),	2.05 (1.54-2.72),
	P = .34	P = .003	P < .001	P < .001
Atypical hyperplasia	0.68 (0.09 to 4.90),	2.57 (1.85 to 3.58),	3.37 (2.58 to 4.40),	5.34 (3.52 to 8.09),
	P = .70	P < .001	P < .001	P < .001

** The hazard ratios are relative to women with nonproliferative breast pathology and scattered fibroglandular densities and are adjusted for age, race/ethnicity, and registry. The P value for interaction between benign breast disease and breast density = 0.28, based on a two-sided Wald test.*

From Tice et al, 2013

PERSONAL HISTORY OF BREAST CANCER

A previous breast cancer increases the risk of cancer in the contralateral breast during a woman's lifetime, ranging from 2 to 11% (Chen *et al.,* 1999). The 10-year risk for invasive breast cancer given a previous in situ carcinoma is 5%. In case of early-stage invasive breast cancer, the observed rates of contralateral breast cancer range between 2.9% and 7.06% at five years (Gao *et al.,* 2003; Li *et al.,* 2015). The annual increase of contralateral breat cancer in women with a previous invasive breast cancer is 1% in premenopausal women and 0.5% in postmenopausal women (Chen*,* 2014).

HORMONAL AND REPRODUCTIVE FACTORS

The main environmental factor that has been linked to breast cancer is the woman's reproductive life, since it runs parallel to the different stages of breast development, through the action of sexual hormones. The various facts of reproductive history (age at menarche, at first birth, at menopause, parity, etc) present different molecular mechanisms, which could result in epidemiological differences: an interesting meta-analysis observed, for instance, that breastfeeding and late age of menarche decrease both ER+ and ER- breast cancer risk, while parity and age at first birth reduce ER+ but not ER- breast cancer risk (Ma *et al.,* 2006).

AGE AT MENARCHE

Menarche gives rise to the start of ovulatory cycles. Proliferation occurs at the end of every luteal phase and, in the absence of pregnancy, mammary epithelial cells undergo apoptosis. The number of ovulatory cycles throughout a woman's life is, therefore, equivalent to the number of times that this epithelium undergoes proliferation and apoptosis and, for the same reason, it is directly linked to premenopausal and postmenopausal breast cancer risk (Clavel-Chapelon *et al.,* 2002). Hence, for each year menarche is delayed, there is a 5 to 20% decrease in breast cancer risk (Hankinson *et al.,* 2008; Chen, 2014), and a case-control study with monozygotic twins showed that, in each pair of twins, the one who had begun menstruating earlier was five times more likely to be diagnosed with breast cancer before her sister (Hamilton *et al.,* 2003).

As previously mentioned, early age at menarche has been observed to increase both ER+ and ER- breast cancer risk (Ma *et al.,* 2006; Chung *et al.,* 2014), with a significantly higher reduction for ER+ breast cancer risk for women with a delayed menarche (Ma *et al.,* 2006).

AGE AT MENOPAUSE

Whereas age at menarche reflects sexual maturity and a significant increase of serum hormone levels, menopause means the activity cessation on the hypothalamus-pituitary-ovary axis and the decrease of hormone levels.

The lifetime number of ovulatory cycles has been traditionally considered a risk factor for breast cancer, so the sooner menarche takes place and the later menopause sets in, the higher the risk.

A late menopause, however, is less determining than an early menarche: for each year menopause is delayed, the risk of breast cancer is increased by 3% (this risk is similar to that due to hormone replacement therapy), while, as mentioned above, for each year menarche is delayed, there is a 5 to 20% decrease in breast cancer risk (Hankinson *et al.,* 2008; Chen, 2014). Actually, Gail model only includes age at menarche and of first birth, but not that of menopause; and, in Hamilton's study, the only relevant effect was that of menarche (neither the age at first birth nor the age at menopause showed a significant effect) (Hamilton *et al.,* 2003).

In addition to this, surgical menopause (bilateral oophorectomy) before the age of 40 (Brinton et al., 1988) or 45 (Trichopoulos et al., 1972) reduces breast cancer risk by half compared to natural menopause after 55 years of age, although this risk reduction has been particularly documented in BRCA1 and BRCA2 carriers (Eisen et al., 2005). Evidence does not show an increased risk of breast cancer after oophorectomy in patients who receive hormone replacement therapy (Rebbeck et al., 2005; Finch et al., 2006; Domchek et al., 2006)

PARITY AND AGE AT FIRST BIRTH

Nulliparous women have 20 to 70% increased risk of breast cancer compared to women with children (Kelsey et al., 1993; Rosner et al., 1994; Colditz et al., 2000), although such increase might be even higher (about 125%) according to a recent meta-analyisis (Namiranian et al., 2014). However, a risk reduction related with child delivery does not appear

until ten years after giving birth (Bruzzi e tal, 1988), and as the number of births increases, so does the protective effect, although more slightly. This fact, however, depends on the mother's age at the time of her first full term birth, so this protective effect disappears after 30 years of age, at which point the risk equals that of a nulliparous woman (Gail et al., 1989; Kelsey et al., 1993; Rosner et al., 1994; Colditz et al., 2000). This difference can be explained by the fact that the first pregnancy represents the major stimulus for mammary development in a woman's lifetime, followed by the highest grade of cell differentiation - epithelial cells which have completed their differentiation remain longer on phase G1 of the cell cycle, where DNA repair takes place (Colditz et al., 1995)- which prevents future cell damage in the young breast, but such damage could be increased by that stimulus in older breasts.

This mechanism might be involved in ER+ rather than in ER- breast cancer, which could explain the reductions in ER+ but not in ER- breast cancer risk associated with this factor (Ma et al., 2006; Ma et al., 2010; Yang et al., 2011; Chung et al., 2014).

This differentiation process requires at least 32 weeks of pregnancy, so abortion, miscarriage and pre-term birth below such gestational age result in partially matured breast tissue, which may not only decrease but could even increase breast cancer risk (Lanfranchi, 2014), although observational studies have not showed an association between abortion or miscarriage and breast cancer risk (see "Miscarriage" section).

A transitory increase in cancer risk exists during the 10 to 20 years following a full term birth, and this is apparently due to the high hormone levels during the postpartum, which could facilitate the progression of a pre-existing neoplasia (Chen, 2014).

Interestingly, an increased risk of ER/PR- or basal-like breast tumors has been observed in association with high parity (Dawood, 2010), but breastfeeding has been observed to eliminate such increase (Gaudet et al., 2011) (see next section).

BREASTFEEDING

Several observational studies, including 2 meta-analyses, have proven a modest duration-dependent protective effect of breastfeeding against breast cancer (Bernier et al., 2000; Tyggvadóttir et al., 2001; Zheng et al., 2001; Collaborative Group on Hormonal Factors in Breast Cancer et al., 2002; Jernströmet al., 2004; Stuebe et al., 2009; Anothaisintawee et al.,

2013), which seems to be more marked in premenopausal cancer and in women with a family history of breast cancer in first-degree relatives (Martin et al., 2005; Stuebe et al., 2009).

A re-analysis of the Collaborative Group on Hormonal Factors in Breast Cancer, which included the results from 47 epidemiological studies with data from more than 50,000 women, estimated a 4.3% reduction in breast cancer risk for each year of breastfeeding and a 7% risk reduction for each birth (Collaborative Group on Hormonal Factors in Breast Cancer, 2002).

Again, the reason for this protective effect seems to rely on the absence of ovulatory cycles, since the breastfeeding period increases prolactin secretion, which inhibits gonadotropin activity and prevents ovulation, which decreases circulating estrogen levels. Moreover, breastfeeding represents the completion of mammary epithelial differentiation and it prevents further influence from carcinogenic changes in the breast (Kelsey et al., 1993). It is noteworthy that, in order for this hormonal pattern to function, breastfeeding must be exclusive and on demand, among other conditions. The high heterogeneity among the types of breastfeeding is responsible for most limitations in the aforementioned studies (Kelsey et al., 1993; Tvggvadóttir et al., 2001; Zheng et al., 2001; Collaborative Group on Hormonal Factors in Breast Cancer et al., 2002; Jernström et al., 2004; Martin et al., 2005; Stuebe et al., 2009).

As to its specific effect on different types of breast cancer, evidence is still inconclusive (Gierarch et al., 2013). Some studies have observed that breastfeeding reduces both ER+ and ER- breast cancer risk (Ma et al., 2006; Phipps et al., 2011), while others suggest it specifically decreases ER/PR- or basal-like tumors, but not ER+ tumors (Millikan et al., 2008; Palmer et al., 2011). There are also data supporting a particular beneficial effect of breastfeeding in women with family history of breast cancer (Stuebe et al., 2009) and in BRCA1 mutation carriers (Kotsopoulos et al., 2012).

MISCARRIAGE AND INDUCED ABORTION

Data from animal studies set the basis for the hypothesis that incomplete mammary cell differentiation during the first trimester of pregnancy could increase their susceptibility to malignant transformation in case of miscarriage (Kelsey et al., 1993). Studies in humans, however, have not provided evidence that miscarriage or abortion modifies breast cancer risk, as shown in the largest cohort study (1,500,000 women) to date (Melbye, 1997). Posterior and smaller studies (Erlandsson et al., 2003; Paoletti et al., 2003; Reeves et al., 2006; Michaels et

al., 2007; Wu et al., 2014), a re-analysis of 53 studies (Beral et al., 2004) and an extensive review (Kitchen et al., 2005) have provided similar data.

ENDOGENOUS HORMONE LEVELS

It has been suggested for decades that sex hormones have a fundamental role in breast cancer. In fact, all reproductive factors explained above, and other which will be discussed later, are associated with cancer through mechanisms that are not completely known, but have in common their dependence on sex hormones.

Given the dramatic differences in sex hormones before and after menopause, a separate analysis will be provided for each hormone with premenopausal and postmenopausal data.

It should be highlighted that, despite the strength of this hypothesis, very few studies assessing this link between urine or serum hormone levels and breast cancer have been conducted, mainly due to their complexity and cost.

ESTROGEN

Estrogen has historically been considered to exert a mitogenic action in the breast at physiological concentration. In fact, there are documents from more than 100 years ago describing the use of oophorectomy to reduce estrogen levels in premenopausal women and prevent breast cancer recurrence (Beatson, 1896). Moreover, as previously mentioned, up to 2 in 3 breast tumors express sex hormone receptors, mainly estrogen and progesterone receptors, and both are basically activated by high levels of estrogen (Lange et al., 2008). For this reason, anti-estrogen therapy is used for hormone-receptor positive breast tumors.

Estradiol is the estrogen found in the highest concentration and with the highest biological activity during the fertile years. It can be identified in serum both free and bound to either albumin or to a sex hormone-binding globulin. Free and globulin-bound estradiol combined represent the fraction with the highest bioavailability for mammary tissue and the highest association with breast cancer risk compared to total estradiol.

From menopause on, estrone and estrone sulfate are the main estrogen compounds, which healthy and malignant breast cells are able to transform into estradiol through sulfatase and 17-ß- dehydrogenase activity (Pasqualini et al., 1996). The source for this estrogen in the postmenopausal woman is no longer the ovary but adipose tissue, so obese postmenopausal women present higher circulating estrogen levels and a higher risk of breast cancer compared with normal-weight postmenopausal women (see "Weight" section).

Besides circulating estrogen levels, the importance of estrogen metabolism has been suggested, since each pathway leads to a compound with specific biological features. This theory, however, has not yet yielded conclusive results (Meilahn et al., 1998; Hankinson et al., 2008).

The possible role of estrogen receptor expression has also been hypothesized, since women belonging to ethnicities with a low breast cancer incidence also present a lower estrogen receptor expression overall (Adami et al., 1998) and, particularly, a lower alpha estrogen receptor expression (ERα) (Lawson et al., 1999). A higher breast cancer risk has been detected in women who overexpress estrogen receptors in the surrounding mammary tissue (Khan et al., 1998).

It should be noted though, that this traditional view of estrogen as a mitogen is currently being discussed, since some groups have identified crosstalk signaling between estrogen and other pathways, resulting in different mechanisms of breast cell death (Rea et al., 2000; Park et al., 2005; Perillo et al., 2008; Perillo et al., 2014).

Regarding epidemiological data, high endogenous estrogen levels have been observed to increase breast cancer risk in both postmenopausal and premenopausal women, with important differences that are discussed below.

POSTMENOPAUSAL WOMEN

The association between estrogen levels and breast cancer risk in postmenopausal women has been found in numerous studies (Lippman et al., 2001; Key et al., 2002; Manjer et al., 2003; Missmer et al., 2004; Kaaks et al., 2005; Beattie et al., 2006; Sieri et al., 2009; Farhat et al., 2011). Further evidence for the role of estrogen in breast cancer risk is provided by the

finding that anti-estrogen therapy (e.g. with aromatase inhibitors) reduces the risk of breast cancer.

One of the aforementioned studies (Lippman, 2001), which evaluated the effect of raloxifen on breast cancer incidence, identified a 2-fold risk increase (RR 2.07) in women presenting estradiol levels equal or higher than 2 pmol/L. That same group underwent the highest risk reduction with raloxifen treatment compared with placebo (79% vs 64%).

A year later, a combined analysis of the 9 prospective studies available at that time was published. It included endogenous estrogen and androgen levels in postmenopausal women (663 case subjects and 1765 control subjects) (Key, 2002). The results (Table 7) show the risk increasing as estradiol levels rise:

Table 7. Association between serum estradiol levels and RR of breast cancer

Estradiol levels (quintiles)	RR (compared with 5th quintile)
1º (highest)	2.0 (1.5-2.7)
2º	1.8 (1.3-2.4)
3º	1.2 (0.9-1.7)
4º	1.4 (1.0-2.0)
5º (lowest)	1.0

From Key et al., 2002

New prospective studies appeared posteriorly, confirming these results (Manjer, 2003; Missmer, 2004; Kaaks, 2005; Farhat, 2011). Missner and Farhat studies particularly found a stronger association between hormone levels and cancer in positive receptor tumors, consistently with the RCTs with SERMs (tamoxifen and raloxifen). Epidemiological studies on obesity and breast cancer risk have also found a higher risk for ER+ tumors.

PREMENOPAUSAL WOMEN

There is less evidence available for premenopausal women, partly due to the fact that hormone concentration during the fertile years presents a higher inter-individual and intra-individual variability. Most studies ignore this important nuance, since they are based on

obtaining blood samples regardless of the day of the cycle. When performing this adjustment, several case-control studies showed an association between high estradiol levels and premenopausal breast cancer (Bernstein and Ross, 1993). Although a large cohort study (Kaaks, 2005) did not identify such association, another cohort study conducted posteriorly established a significant link between total and unbound estradiol during follicular phase and breast cancer risk (Eliassen, 2006): women at the top quartile were at least twice more likely to develop breast cancer compared to women at the lowest quartile (RR 2.1 for total estradiol and RR 2.4 for unbound estradiol). Again, this association proved itself stronger in ER+ tumors (RR 2.7 for total estradiol and 2.8 for unbound estradiol). A recent study analysing the association between the levels of different estrogenic compounds at different points of the cycle and the ocurrence of breast cancer found no association between follicular estradiol, follicular estrone, follicular free estradiol, luteal estrone, luteal free estradiol or progesterone and risk of either total or invasive, premenopausal or postmenopausal breast cancer. High levels of luteal estradiol, however, were linked to 70% higher risk of hormone receptor positive breast tumors (Fortner *et al.,* 2013).

ANDROGEN

POSTMENOPAUSAL WOMEN

Most (Cauley *et al.,* 1999; Key *et al.,* 2002; Missmer *et al.,* 2004; Kaaks *et al.,* 2005; Eliassen *et al.,* 2006; Tworoger *et al.,* 2006; Cummings *et al.,* 2009; Dorgan *et al.,* 2010) but not all studies (Wyoswoski *et al.,* 1987; Thomas *et al.,* 1997) have found an association between androgen levels and postmenopausal breast cancer, particularly ER+.

A dual effect of testosterone in breast carcinogenesis has been suggested, leading to proliferation when binding to estrogen receptors or when converting to estrogen through aromatization (Bernstein and Ross, 1993; Brettes *et al.,* 2008) and inhibiting such proliferation when binding to androgen receptors.

Two combined analyses (Key 2002, Kaaks 2005) showed an increased breast cancer risk for testosterone levels when comparing the highest quintile to the lowest quintile (RR=2.2), with similar results for other androgen compounds. When adding estradiol levels for risk calculation, a very slight decrease was found, suggesting an estrogen-independent role of

androgen. It is important to remark that this effect is practically limited to hormone receptor positive tumors (Kaaks *et al.,* 2005; Eliassen *et al.,* 2006; Tworoger *et al.,* 2006; Dorgan *et al.,* 2010). In a recent case-control study, high testosterone levels did not increase but they significantly lowered the risk of hormone receptor negative breast cancer in postmenopausal women (Farhat *et al.,* 2011).

PREMENOPAUSAL WOMEN

As occurs with estrogen, data in premenopausal women are not as abundant as with premenopausal women. However, several nested case-control studies (Thomas *et al.,* 1997; Micheli *et al.,* 2004; Kaaks *et al.,* 2005; Eliassen *et al.,* 2006; Dorgan *et al.,* 2010) demonstrated a similar association between high androgen levels and ER+ premenopausal breast cancer risk (RR=1.5).

PROGESTERONE

The role of progesterone in breast physiology and its ability to induce tumorogenesis in rodents have been known for more than thirty years (Kelsey *et al.,* 1979). Its association with cancer has yielded, however, contradicting results: on one hand, a risk reduction due to the balancing of estrogen effect in the breast has been supported (Kelsey, 1979); on the other hand, a risk increase has been identified, through induction of mitosis in the late luteal phase (Bernstein and Ross, 1993).

Some previously mentioned large prospective studies have yielded heterogeneous and inconsistent results (Micheli *et al.,* 2004; Kaaks *et al.,* 2005; Eliassen *et al.,* 2006), partially due to the difficulty of hormone determinations.

PROLACTIN

The observation that more than 50% breast tumors expressed prolactin receptors (Partridge and Hähnel, 1979) suggested a role for prolactin in breast cancer. In vitro studies also found a positive effect of prolactin on proliferation, survival, motility and vascularity. In animals, it increased tumor growth rate and the number of metastasis.

POSTMENOPAUSAL WOMEN

Prolactin determination in women is difficult and highly unreliable, since stress –both physical and emotional- can alter its levels, which does not allow a reliable measure after cancer diagnosis. Nevertheless, the few available studies seem to find a slight association (RR=1.4) between high prolactin levels and breast cancer, particularly postmenopausal breast cancer (Hankinson *et al.,* 1999; Tworoger *et al.,* 2004; Tworoger *et al.,* 2006). This last study showed a differential effect of prolactin according to the hormone receptor pattern (Table 8):

Table 8. RR of breast cancer by hormone receptor expression pattern

	RE+/RP+	RE+/RP-	RE-/RE-
RR (superior vs inferior quartile)	**1.8**	**1.9**	0.8

From Tworoger, 2006

The role of prolactin in hormone receptor positive tumors was also demonstrated in a study with transgenic mice, in which high prolactin levels stimulated both ER- and ER+ tumors (Rose-Hellekant *et al.,* 2003).

PREMENOPAUSAL WOMEN

The scarce data on premenopausal women suggest an association between prolactin and cancer. Although Tworoger *et al.* (Tworoger *et al.,* 2006; Tworoger *et al.,* 2007) observed similar results in premenopausal and postmenopausal women, more recent publications -including a 20-year prospective study- have not found any effect (Berinder *et al*., 2011; Tworoger *et al.,* 2013).

INSULIN AND RELATED HORMONES

Abundant evidence, from both in vitro and in vivo studies, suggests that metabolic disturbances have an adverse effect on breast cancer cell survival and progression (Zielinska *et al.,* 2015). Breast cancer patients with metabolic syndrome have been found to present more aggresive tumors (Healy *et al.,* 2010) and various studies with mice have established a link between high-energy and high-glucose diets with tumor growth, increase in the number of metastases and resistance to chemotherapy (Phoenix *et al.,* 2010; Zeng *et al.,* 2010).

IGFis a protein hormone, structurally analogous to insulin, which exerts a series of cellular effects under the influence of insulin, growth hormones, nutrition and systemic disease status, including cancer (Zielinska *et al.,* 2015). However, each individual, even each individual tissue has a set of locally expressed component of the IGF family, which accounts for the variety of actions of IGF. The fact that IGF is metabolically regulated and mediates the effects of nutition on cell growth (Hursting *et al.,* 2010) is supported by the evidence on the role of IGF-1 in metabolic changes in tumor growth and survival (Zielinska *et al.,* 2015). As in other kinds of tissues, the IGF-1 axis can promote normal and cancer breast epithelial cell proliferation (Pollak *et al.,* 1998). Renehan's meta-analysis in 2004 (Renehan *et al.,* 2004) observed the association between insulin concentration and breast cancer, particularly in premenopausal women (RR 1.65 75^{th} vs 25^{th} percentile), and he also found a link between the level of an IGF-binding protein (IGFBP-3) and premenopausal breast cancer risk (RR 1.51, 75^{th} vs 25^{th} percentile). Although posterior prospective studies have yielded contradictory evidence, a recent combined study, including 17 prospective studies (Endogenous Hormones and Breast Cancer Collaborative Group, 2010) has linked high IGF-1 levels to premenopausal and postmenopausal breast cancer risk.

Circulating insulin levels are more difficult to measure and very few studies include them. However, when evaluated, a significant relation between high insulin levels and breast cancer in postmenopausal women without diabetes mellitus and without hormone replacement therapy (Gunter *et al.,* 2009). Interestingly, the association between obesity and breast cancer risk –which will be further discussed- weakens after adjusting by insulin concentration, which suggests a preminent role of hyperinsulinemia, together with estrogen, in that association. However, it has not been yet established that diabetes mellitus represents a risk factor for breast cancer (Wolf *et al.,* 2005), due to contradictory study results (Chen, 2014; García-Esquinas *et al.,* 2015).

BONE DENSITY

Given the bone sensitivity to estrogen action and expression and estrogen receptors, bone density can be considered an indirect marker for long-term endogen estrogen exposure. Several studies have linked an increased bone density with a higher breast cancer risk (Cauley *et al.,* 1996; Zhang *et al.,* 1997; Zmuda *et al.,* 2001; Chen *et al.,* 2008; Qu *et al.,* 2013). A recent meta-analysis including 70,878 postmenopausal women concluded that women with highest

hip bone mineral densities (BMD) were 62 percent more likely to develop breast cancer compared with women in the lowest BMD category (RR 1.62, 1.172.06, p <0.001) (Qu *et al.,* 2013).

BREAST DENSITY

Breast density varies considerably across the population and is determined by hereditary factors (Boyd *et al.,* 2002; Vachon *et al.,* 2007) and also by modifiable environmental factors (Boyd *et al.,* 1997; Irwin *et al.,* 2007), including hormonal treatment: breast density is increased by hormone replacement therapy and it is reduced by tamoxifen.

The main problem consists in the difficulty for mammograms to detect tumors in dense breasts. However, the very existence of dense breast tissue is an independent risk factor for breast cancer (Boyd *et al.,* 1995, 2005 y 2007; McCormack *et al.,* 2006; Barlow *et al.,* 2006; Wong *et al.,* 2011; Pollán *et al*., 2013): women with radiologically dense breasts present a 3-fold to 6-fold higher cancer risk when compared with women with normal-density breasts.

However, there is some controversy about the association between mammographic density and estrogen activity. While some authors suggest that mammographic density might be the result of endogenous hormonal exposure (Daye *et al.,* 2013)

While some authors have observed how breast density increases breast cancer risk, regardless of hormone receptor status (Ziv, 2004), some data support a higher incidence of estrogen receptor negative tumors (Yaghjyan, 2011). And more recently, an interesting study has found that the combination of breast density and breast texture (a new image-derived marker) correlate with a higher risk of estrogen receptor positive tumors (Keller *et al.,* 2014).

From the chemical, circulating sex hormone levels are not correlated to breast density (Tamimi *et al.,* 2007). This might be due to the fact that breast density depends on other growth factors, such as IGF-1, although this hypothesis has not been properly proven so far (Verheus *et al.,* 2008).

EXOGENOUS HORMONAL FACTORS

It is crucial to differentiate between premenopause and postmenopause when analysing the effect of exogenous hormone use. While oral contraceptives and ovulation inducers used during the fertile years are only slightly associated with an increased risk of

breast cancer (Gierisch *et al*., 2013), hormone replacement therapy in postmenopausal women, especially long-term treatment, shows a clear increase of breast cancer risk (Chen *et al.,* 2015).

ORAL CONTRACEPTIVES

Before discussing the different findings about the link between oral contraception and breast cancer, two scenarios must be considered: current or recent use of oral contraceptives and past use of oral contraceptives –particularly in postmenopausal women, but not exclusively.

A large amount of epidemiological studies, including three large cohort studies and a meta-analysis, have evaluated long-term exposure to oral contraceptives and breast cancer risk, without having found a significant increase (The CDC Cancer and Steroid Study, 1983; The Cancer and Steroid Hormone Study of the CDC and the NICHD, 1986; Hankinson *et al.,* 1997; Marchbanks *et al.,* 2002; Davidson *et al.,* 2002; Vessey *et al.,* 2006; Hannaford *et al.,* 2007; Moorman *et al.,* 2013; Vessey *et al.,* 2013). Consistently, the combined analysis from 1996 (Collaborative Group on Hormonal Factors in Breast Cancer, 1997) which included more than 50000 breast cancer patients and more than 100000 healthy women, could not establish such association either. There is a very interesting data splitting between premenopausal and postmenopausal women: the former did not experience any risk increase, whereas the latter seem to present a higher breast cancer risk (RR 1.4-1.5) following long-term contraceptive use (Romieu, 1990; Thomas, 1991), especially before 35 years of age. The same finding has been observed in several case-control studies and it might be attributed to a fast risk reduction after discontinuation of contraceptives.

However, that same combined analysis identified a higher incidence of breast cancer in current or recent contraceptive users (RR 1.24) and, interestingly, women who had discontinued contraceptive use at least ten years earlier had the same risk as never users. When analysed jointly, duration of treatment and time lapse since last dose, the risk increase was found to be restricted to women who were using contraceptives at the time of the study or women who had recently interrupted its use, regardless of duration, even in younger women. This influence of recent versus long-term use could suggest a late cancer promoter role of contraceptives. Other studies contradict this hypothesis and find no significant

association between oral contraceptives and breast cancer risk, irrespective of the timing, duration, age of initiation and estrogen dose or race (Marchbanks *et al.,* 2002).

An old hypothesis suggests that oral contraceptive use leads to higher breast cancer risk before the first full term birth (Russo *et al.,* 1990) –when the highest mammary development is reached- than after the first full term birth. This has led to further study the effect of contraception in nulliparous women, with a slight risk increase after prolonged use in the first (Romieu *et al.,* 1990; Thomas *et al.,* 1991).

Evidence on breast cancer risk in oral contraceptive users with a family history of breast cancer is also conflicting. Studies considering current dosage have not found an increased risk in women with a family history of breast cancer (Marchbanks *et al.,* 2002; Moorman *et al*., 2013), while studies based on older data, with high-dose formulations, have even shown 3-fold higher breast cancer risk (RR 3.3, 95% CI 1.6-6.7) (Grabick *et al.,* 2000). In fact, differences in contraceptive pill composition have not been taken into account in most studies. Use of progestin-only contraceptives, in their various presentations, has so far proven to equal or even decrease breast cancer risk, in comparison with no use (Waaler and Lund, 1983; Standford and Thomas, 1993, Marchbanks, 2002). This information must, again, be cautiously interpreted, since new low-dose preparations have been commercialized since their publication.

Regarding BRCA mutations, data are still confusing: while some authors have observed no risk increase in mutation carriers who use oral contraceptives (Moorman *et al.,* 2013), others have found different effects depending on the type of study. For instance, Friebel *et al*. (Friebel *et al*., 2014) did not detect any effect of oral contraceptives in case-control studies, but a higher risk of breast cancer was found in BRCA1 and BRCA2 mutation carriers in cohort studies.

HORMONE REPLACEMENT THERAPY (HRT)

Consistently with the findings on estrogen and breast cancer risk, much of the available evidence supports a causal relationship between HRT and breast cancer. Observational studies have shown an increased risk of breast cancer with HRT, either unopposed estrogen therapy or combined estrogen-progestin therapy (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Beral *et al.,* 2003). A re-analysis found that for each

year a woman uses HRT, her risk of breast cancer increases by 2.3 percent (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Definitive evidence of the effects of HRT was provided by the the randomized, placebo-controlled Women's Health Initiative (WHI) trial (Rossouw *et al.,* 2002), which had to be due to higher incidence of breast cancer, and adverse cardiovascular events, with no evidence of overall health benefit.

Given the differences observed according to the type and duration of HRT, and the existence of data provided by a large clinical trial, specific results discussed separately.

ONLY ESTROGEN

OBSERVATIONAL STUDIES

The first obvious limitation to study HRT effect on postmenopausal breast cancer risk is that treatments have considerably changed since the first estrogen-only preparations began to be used several decades ago. The most comprehensive analysis performed to date (Collaborative Group on Hormonal Factors in Breast Cancer, 1997) shows higher breast cancer risk in current or recent users of HRT, which increases with duration of treatment (Table 9), while women who discontinued HRT use for the last 5 years or longer did not present higher risk, regardless of duration.

Table 9. Association between duration of only-estrogen HRT and risk of invasive breast cancer

Years of treatment	RR (versus non-users)
1-4	1.08
5-9	1.31
10-14	1.24
15 or more	1.56

From Collaborative Group on Hormonal Factors in Breast Cancer, 1997

Preparations with unopposed estrogen have later proven to increase breast cancer risk as long-term treatments, but not when used for short periods of time (Beral, 2003; Chen,

2006). The increase is particularly significant after 15 years of use, especially for hormone receptor positive tumors (Chen, 2006).

Although the link between obesity and breast cancer will be discussed later, an important remark must be highlighted: the combined analysis from 1997 and other posterior studies have observed how HRT for longer than 5 years does increase breast cancer risk in normal-weight patients. This might be related with the lower circulating estrogen levels observed in these women, so exogenous administration leads to a higher relative increase in total estrogen levels compared with obese women.

WHI

In contrast with evidence from most (Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Beral *et al.,* 2003) but not all (Li *et al.,* 2003) observational studies, the WHI found a slightly lower risk of breast cancer in the group taking unopposed estrogen when compared with the placebo group after a mean follow-up of 7.1 years (HR 0.80, 95% CI 0.62-1.04) (Stefanick *et al.,* 2006; Prentice *et al.,* 2008). However, women on unopposed estrogen were more likely to present abnormal mammograms.

COMBINATION THERAPY

OBSERVATIONAL STUDIES

Growing evidence that linked only-estrogen preparations with endometrial hyperplasia and cancer in the 1980's prompted combination HRT with both estrogen and progestin. Despite leading to a significant reduction of those conditions, this regimen was found to increase breast cancer risk to a higher extent than only-estrogen preparations (Bergkvist, 1989; Collaborative Group on Hormonal Factors in Breast Cancer, 1997; Colditz and Rosner, 2000). For instance, the Collaborative Group found a 50% risk increase for 5 or more years of combined HRT (RR=1.53) and a 30% risk increase for only-estrogen (RR=1.31). Some large observational studies and a big-scale clinical trial conducted later have yielded similar results (Table 10):

Table 10. Results from different observational studies and one clinical trial on the effect of HRT and breast cancer incidence

Study	Type	Duration of exposure	RR (E+P**)	RR (E*)	HR (E+P**)	RR (E+T***)
Schairer *et al.,* 2000	Cohort	4 years, current or recent	2.0	1.0		
Colditz y Rosner, 2000	Cohort	10 years	1.67	1.23		
Million Women Study. Beral *et al.,* 2003	Cohort	Current use	2.0			
Magnusson *et al.,* 1999	Case-control	Long duration Cyclic Continous	3.0 5.4 2.4			
WHI. Chlebowski *et al.,* 2003	RCT	7 years			1.24	
Tamimi *et al.,* 2006	Cohort					2.48

Only-estrogen. ** Estrogen + progestin. * Estrogen+ testosterone.*

From Hankinson, 2008

WHI

In the WHI combination estrogen-progestin (conjugated equine estrogens 0.625 mg and medroxyprogesterone acetate 2.5 mg) arm, the risk of invasive breast cancer was significantly increased with combined therapy after an average follow-up of 5.6 years (HR 1.2) compared with placebo (Chlebowski *et al.,* 2003). However, such increase was not observed until 3 years of treatment in previous HRT users, and not until 4 years of treatment in new users. Regarding the attributable risk there were eight excess cases per 10,000 person-years at an average of 5.2 years (Rossouw *et al.,* 2002).

Interestingly, data gathered after stopping treatment showed a nonsignificant increase in breast cancer risk after an additional mean follow-up of 2.4 years after stopping the WHI trial (mean HR over the follow-up period 1.27, 95% CI 0.91-1.78) (Heiss *et al.,* 2008). However, this finding still lacks clinical relevance and is not supported by abundant evidence from observational studies (Collaborative Group on Hormonal Factors in Breast Cancer, 1997).

In a more detailed analysis of breast cancer risk in the postintervention phase of the WHI (combination therapy versus placebo) and the observational cohort (ever users of HRT

versus non-users), a significant decrease in breast cancer incidence was observed after stopping treatment (Chlebowski *et al.,* 2009).

EFFECT OF PROGESTINS

The hypothesis that progestins increase breast cancer risk is based in two parallel facts. Firstly, the highest breast proliferation in premenopausal women takes places during the luteal phase, when progesterone reaches its peak Clarke and Sutherland, 1993). Thus, progestins might increase cell division in the breast, leading to accumulated DNA errors that eventually result in breast cancer or in a greater proliferation of malignant cells (Colditz *et al.,* 1998). Secondly, observational studies including women treated with combined therapy and unopposed estrogen have shown a greater increase in mammographic density (Greendale *et al.,* 1999) and more cell proliferation in benign breast biopsies (Hofseth *et al.,* 1999) in the former group. In the latter study, breast proliferation was located in the terminal duct-lobular unit, an area where most breast cancers develop. However, in vivo (Cline *et al.,* 1996) and in vitro studies (Wren, 1995) have found differing effects of progesterone on breast cell proliferation.

OBSERVATIONAL STUDIES

Many of the earlier epidemiologic studies have grouped together estrogen-only and combined therapy users. However, when distinguishing between these groups, most studies have found combined therapy to present a greater risk of breast cancer compared with unopposed estrogen (Schairer *et al.,* 2000; Ross *et al.,* 2000; Beral *et al.,* 2003; Li *et al.,* 2003; Olsson *et al.,* 2003; Stahlberg *et al.,* 2004; Fournier *et al.,* 2005).

In the Million Women Study, current use of HRT was associated with an increased risk of breast cancer (RR 1.30, 95% CI 1.2-1.4; and RR 2.0, 95% CI 1.88-2.12 for unopposed estrogen and combined therapy, respectively) (Beral *et al.,* 2003). There has also been suggested that progestin given continuously with estrogen may be associated with higher risk than regimens in which the progestin is given in a cyclic fashion (Tjonneland *et al.,* 2004; Bakken *et al.,* 2011; Cordina-Duverger, 2013) but this has not been reported in all studies (Ross *et al.,* 2000; Beral *et al.,* 2003). In a prospective cohort study of approximately 80,000 women, menopausal hormone regimens containing estrogen plus a synthetic progestin were associated with an excess breast cancer risk, while regimens containing estrogen plus natural progesterone were not (Fournier *et al.,* 2008). Consistenly with this observation, a recent case-control study

(Cordina-Duverger *et al.,* 2013) found no increased breast cancer risk among combined therapy users treated with natural micronized progesterone, but it showed that progesterone-derived compounds increased breast cancer risk by more than half (OR 1.57, 0.99-2.49 CI) and testosterone-derived compounds by more than 3-fold (OR 3.35, 1.07-10.4 CI).

WHI

As previously explained, the WHI study included a group on combined therapy (conjugated equine estrogens 0.625 mg and continuous medroxyprogesterone acetate 2.5 mg), a group on unopposed estrogen and a placebo group. When comparing the two first groups, an increased risk in the arm receiving combination therapy was detected: HR 1.24, CI 1.02-1.50 vs HR 0.80, CI 0.62-1.04 (Prentice, 2014). This finding supports previous evidence that progestins (particularly medroxyprogesterone acetate in a continuous fashion, which was the chosen compound) added to breast cancer risk, compared with estrogen alone.

DURATION OF USE

Most observational studies do not find an increased risk of breast cancer in women who receive combined therapy for less than four or five years, but they do detect an increase with longer duration of use (Colaborative Group on Hormonal Factors in Breast Cancer, 1997; Li *et al.,* 2003). According to their findings, the relative risk for developing breast cancer was 1.35 for women who were current hormone users and had taken hormones for five years or longer compared with never users (Colaborative Group on Hormonal Factors in Breast Cancer, 1997). The WHI yielded a similar time, although the increase in risk was seen after only three years in women who had previously used menopausal hormones (Chlebowski *et al.,* 2003).

There did not appear to be increased risk of breast cancer in the WHI for women on unopposed estrogen. However, in an updated report from the Nurses' Health Study of 28,835 women who had undergone hysterectomy, long-term use of unopposed estrogen was associated with a statistically significant increase in breast cancer risk (RR for current use >20 years = 1.42, 95% CI 1.13-1.77) (Chen *et al.,* 2006). The risk of ER+/PR+ cancers became statistically significant after 15 years of unopposed estrogen use (RR 1.48, 95% CI 1.05-2.07), with similar results reported by another prospective cohort study (Zhang *et al.,* 2007). Results from the Million Women Study also reported an increased breast cancer risk with longer duration of unopposed estrogen. However, that study found an increased risk of breast cancer

with unopposed estrogen use for less than five years, in contrast to most data, including the WHI.

Interestingly, while duration of use seems to modify breast cancer riks for current HRT users, this finding has not been observed for past users. In the combined analysis of epidemiologic studies, women who had stopped HRT more than five years before were not at increased risk compared with never users, irrespective of the duration of use (Colaborative Group on Hormonal Factors in Breast Cancer, 1997). However, there have not been enough data on long-term past users; there may still be a risk associated with past use if the duration was long enough (Chen, 2014).

TIMING OF HORMONE REPLACEMENT THERAPY

It is still unclear whether the benefits and risk of HRT vary with time of initiation relative to women's age at menopause. For cardiovascular disease, some data suggest that women who start closer to the time of natural menopause may benefit from treatment to a greater extent than those who start later (Hodis and Mack, 2014). However, limited data on breast cancer suggest that women who start therapy around the time of menopause may be at greater risk of breast cancer than those who start later after menopause (Beral *et al.,* 2011; Chen, 2014), regardless of the type of therapy (combined or estrogen-only). Consistently with these, the WHI also observed greater breast cancer risk with initiation of combined therapy around the time of menopause (Chlebowski *et al.,* 2010; Prentice *et al.,* 2008). However, most studies lack adjusting for the fact that women who start treatment closer to age at menopause generally have longer durations of use.

HORMONAL CHEMOPREVENTION

Another proof of the role of hormones in breast cancer –which also represents a treatment option in specific cases- is the existence of SERM (selective estrogen-receptor modulators). These drugs present agonist or antagonist activity depending on the tissue where they exert their actions. The best known SERM is tamoxifen (McDonnell, 2000; McDonnell *et al.,* 2001; Park and Jordan, 2002; Stygar *et al.,* 2003).

There is long-term experience with tamoxifen as a therapeutic resource in ER+ breast tumors and its prophylactic effect has been studied for some years in healthy women at high risk of developing breast cancer (Table 11). Preclinical studies have found that SERMs are able

to antagonize 17-ß-estradiol-mediated stimulation of malignant epithelial cell proliferation in the breast (Shang and Brown, 2002; Miller, 2002; Smith and Taylor, 2002; Hemachandra *et al.,* 2014). It is important to remark that, unfortunately, tamoxifen treatment increases thromboembolyc events and endometrial hyperplasia and endometrial cancer, due to its agonist effect in the endometrium (Zujewski, 2002). Raloxifen is a more recent SERM that seems to present a higher safety profile, given its neutral effect on the uterus (Zujewski, 2002; DeMichele *et al.,* 2008).

Table 11. Breast cancer risk reduction with SERM and AI use in different studies

Study	Type	Follow-up	RR breast	RR endometrium	RR VTD
Fisher *et al.,* 1998	RCT (tamoxifen vs placebo)	69 months	0.51	2.53	*Not significant*
IBIS-I. Cuzick *et al.,* 2002 and 2015	RCT (tamoxifen vs placebo)	16 years (as per published long-term follow-up results)	0.71	*Not significant*	2.5
NSABP Fisher *et al.,* 2005	RCT	7 years	0.57	3.28	*Not significant*
STAR. Vogel *et al.,* 2006	RCT (raloxifen vs tamoxifen)	5 years	1.02	*Not significant*	0.70
RUTH. Barret-Connor *et al.,* 2006	RCT (raloxifen vs placebo)	5.6 years	0.56	-	1.44
IBIS-II Cuzick *et al.,* 2014	RTC (anastrozole vs placebo)	5 years	0.47	*Not significant*	*Not significant*

VTD: venous thromboembolic disease.

Modifed from Hankinson, 2008

Beyond SERM, there is evidence on the role of other hormone medications in reducing breast cancer risk. Aromatase inhibitors (AI), which had already showed a higher efficacy than tamoxifen in treating ER+ tumors, also reduce contralateral breast cancer risk in postmenopausal women (Gradishar, 2006). Another AI, exemestane, had been studied earlier for breast cancer prophylaxis in high-risk women, with a 65% reduction of invasive breast cancer incidence (Goss *et al.,* 2011). However, the follow-up period was too short to rule out serious adverse effects (Cuzick *et al.,* 2014).

In addition to this, increasing although contradicting evidence has been published about the effect of somatostatin and its analogues in breast cancer. Their anti-proliferative effect on both normal and tumor cells *in vitro* has already been demonstrated (Bousquet *et al.,* 2001), as well as their role as regulators of hormone secretion, through reduction of circulating

levels of GH and IGF-1 (Bevan *et al.,* 2002). Furthermore, there are data that support an improved survival rate for metastatic breast cancer patients who received somatostatine analogues in addition to standard treatment (Bontenbal *et al.,* 1998; Dolan *et al.,* 2001; Pollack *et al.,* 2010). Despite the need for further evidence in this field, it has been hypothesized that treatment with somatostatin analogues might reduce breast cancer incidence (Pollack, 1998; Frati *et al.,* 2011).

ETHNICITY

The highest rates of breast cancer in the United States are found in Caucasian women (124 cases/100000 women-year), while the lowest rates are found in African-American women (113 cases/100000 women-year), native American (92 cases/100000 women-year), hispanic (90 cases/100000 women-year) and Asian (82 cases/100000 women-year) (American Cancer Society, 2009-2010).

Although ethnicity-related risk has still few global relevance in Spain, physiopathological mechanisms explaining those differences might be interesting, as in African-American women. These women present a lower incidence of breast cancer compared to those from European-ascent, but with an earlier onset and a more aggressive course of disease, including a higher rate of hormone receptor negative tumors and basal-like carcinomas. Specific BRCA1 and BRCA2 mutations have been identified for this group (Isaacs, 2011).

LIFESTYLE AND DIET

SOCIOECONOMIC STATUS

Belonging to a high socioeconomic status nearly duplicates breast cancer risk, although this does not seem to represent an independent risk factor but the result of others: diet, reproductive patterns, access to health care, etc (Hankinson, 2008).

GEOGRAPHICAL RESIDENCE

North America, Australia, New Zealand and Western and Northern Europe present the highest breast cancer rates, while the lowest incidence is found in Asia and Subs-Saharan Africa. Interestingly, incidence is decreasing in high-risk areas and it is increasing in low-risk areas (Ferlay *et al.,* 2015), probably due to emerging environmental factors linked to industrial

development, some of which have already been discussed: fat intake and body weight, age at menarche and reproductive habits. The most obvious expression of this theory is observed in women belonging to a low-risk ethnicity or coming from a low-risk area. When these women emigrate and arrive in a high-risk country, their descendants reach the same breast cancer risk as women from that high-risk area after one or two generations (Willett *et al.,* 2004).

ANTHROPOMETRICS

WEIGHT

The role of weight on breast cancer is rather complex and presents contradicting effects depending on menopausal status. The growing incidence of obesity and overweight in Western and developing societies has also been observed within breast cancer patients: in a study of over 1000 women treated for breast cancer at the MD Anderson Cancer Center in Houston, 30% were found to be obese and 32%, overweight (Litton *et al.,* 2004). A Spanish study in 2010 found even higher proportions of obese (48%) and overweight (34%) patients (Amaral *et al.,* 2010).

POSTMENOPAUSAL WOMEN

Elevated BMI or body weight increases postmenopausal breast cancer risk (van den Brandt *et al.,* 2000; Morimoto *et al.,* 2002; Feigelson *et al.,* 2004; Lahmann *et al.,* 2004; Eliassen *et al.,* 2006; Ahn *et al.,* 2007), especially in women who do not use HRT (Eliassen *et al.,* 2006). BMI ≥33 represents a 27% higher risk of breast cancer compared to BMI ≤21 (van den Brandt *et al.,* 2000). The underlying mechanism explaining this difference seems to rely on the conversion of estrogen precursors to estrogen in adipose tissue, which leads to a rise in estradiol –the most biologically active estrogen- and a reduction of sex hormone binding globulin. This reaction is specifically linked to abdominal adiposity. High insulin levels must also be considered, since they are frequently found in obese patients and they have proved to be an independent risk factor for breast cancer (Gunter *et al.,* 2009).

PREMENOPAUSAL WOMEN

In premenopausal women, this association is inverted: BMI ≥31 decreases breast cancer risk by 46% compared to BMI ≤21 (van den Brandt *et al.,* 2000). A possible explanation

for this fact is that, during the fertile years, obesity leads to anovulation through various mechanisms, which leads to reduced levels of estrogen and progestin. However, other cycle-independent factors must necessarily exist, since the association between BMI and cancer remains unchanged when adjusted by menstrual pattern.

HEIGHT

Height has been linked for decades with a slightly increased risk of breast cancer. The influence of insulin and IGF-1 levels is directly proportional to height. Classic studies linking height and breast cancer were performed in populations in which reduced height was a reflection of low body weight and poorer nutrition, which led to hypothesize that the association between height and cancer was confounded by weight (Tretli *et al.,* 1989; Vatten y Kvinssland *et al.,* 1992). However, more recent studies contradict this theory and suggest that height is an independent risk factor (Kabat *et al*., 2013; Kabat *et al*., 2014).

PHYSICAL ACTIVITY

This factor presents evident quantification difficulties and seems to exert opposite effects before and after menopause. In premenopausal women, intense exercise is linked to anovulation, which in turn leads to lower risk. However, regular exercise is also associated with lower body weight, which increases premenopausal breast cancer risk. A case-control study which accurately evaluated the amount of physical activity in premenopausal women determined that 3.8 hours of moderate exercise weekly reduced cancer risk by 58% (Bernstein *et al.,* 1994). The effect of exercise on postmenopausal women is unequivocally protective and it seems to be related to lower circulating estrogen levels, but not to BMI (Lee *et al.,* 2001; McTiernan *et al.,* 2004; Monninkhof *et al.,* 2009; Friedenreich *et al.,* 2010).

DIET CONTENT OF SPECIFIC NUTRIENTS

Alcohol is the only diet component that has been consistently associated with breast cancer risk, with a dose response relationship that can be observed in both low and high consumption levels (Singletary *et al.*, 2001; Zhang *et al.,* 2007; Allen *et al.,* 2009; Chen *et al*., 2011; Bagnardi *et al.,* 2013), with a 10% risk increase with each 10 g of daily alcohol intake (Chen *et al*., 2011). Interestingly, this effect of alcohol appears restricted to specific hormone-receptor patterns. The WHI trial observed an 11% risk increase with each 10 g of daily alcohol intake for ER+/PR+ breast cancer, but not for ER+/PR- or ER-/PR- breast cancer (Zhang *et al.,*

2007). Similarly, a large cohort study recorded a 63% increased risk of ER+/PR+ breast cancer with a high alcohol intake, with no influence on ER+/PR- or ER-/PR- breast cancer (Falk *et al.,* 2014).

The biological basis for the effect of alcohol on breast carcinogenesis includes higher serum levels of sex hormones, direct DNA damage in breast cells, increased breast cell susceptibility and a higher invasive potential (Singletary *et al.*, 2001).

Moreover, some factors have been observed to modify the effect of alcohol on breast cancer risk. The Nurses' Health Study showed a similar effect (30% risk increase) of either alcohol or hormone replacement therapy in breast cancer risk, but a significantly higher effect for the combination of both factors (100% risk increase) (Chen *et al.,* 2002). Conversely, folic acid intake seems to lessen the risk increase attributed to alcohol (Zhang *et al.,* 1999; Choumenkovitch *et al.,* 2002; Zhang *et al*., 2003).

The influence of multiple substances on breast cancer has been analysed (fibre, carotenoids, vitamin A, vitamin E, vitamin C, selenium, folates, calcium, vitamin D, phytoestrogen, antioxidants, caffein) with inconclusive results and very modest risk reductions.

EXPOSURE TO IONIZING RADIATION

A clear association between moderate to high levels of ionizing radiation and breast cancer risk has been demonstrated, through data obtained from women exposed to the effect of the atomic bombs in Japan during the Second World War, TBC patients repeatedly subjected to fluoroscopy, and Hodgkin lymphoma patients treated with radiation therapy at an early age (John *et al.,* 1993; Kenney *et al.,* 2004; Guibout *et al.,* 2005; Pukkala *et al.,* 2006; Henderson *et al.,* 2010). Risk is maximal when radiation is received before puberty (RR ≅9) and it persists when exposure takes place before 45 years of age, but after that no higher risk has been observed.

Regarding radiation use for diagnostic purposes, no association with breast cancer has been found.

LIGHT AT NIGHT AND SHIFT WORK

An increase in breast cancer risk by almost 50% has been found in women exposed to artificial light during night-time hours, especially night-shift workers (RR 1.48) (Megdal *et al.,*

2005). The suggested underlying mechanism consists in a decrease of melatonin levels. A reduction in breast tumor growth has been observed in rodents upon administration of melatonin. Moreover, extirpation of the pineal gland, which leads to disruption of melatinonin secretion, induces rapid tumor growth (Tamarkin *et al.,* 1981). IARC has recognised night work as a human carcinogen (Streif *et al.,* 2010).

INFECTION

Epstein-Barr virus has been identified in 20 to 46% of breast cancer tissue samples. Human mammary tumor virus (HMTV) was discovered in 1998, after being identified in 37% of almost 400 analysed samples (Wang, 1995). This virus is genetically similar to murine mammary tumor virus (MMTV), found by Bittner in 1996. Data retrieved in the past 15 years suggested a possible pathogenic role for MMTV in breast cancer, based on its higher presence in breast tumors as compared to healthy tissue, within the same patient (Labat 1998; Melana *et al.,* 2010; Nartey *et al.,* 2014). Highest presence of the virus has been identified in gestational, inflammatory and aggressive tumors, which has, in turn, led to suggest an association of the virus with hormonally responding tissues (Nartey *et al.,* 2014). In fact, HMTV has been identified in endometrial carcinomas (Deligdisch *et al.,* 2013). However, other studies have not shown difference between the presence of HMTV in affected and healthy tissues from the same patient (Cedro-Tanda *et al.,* 2014).

IN UTERO EXPOSURES

Several *in utero* exposures have shown an association with breast cancer risk: high weight at birth, being firstborn, maternal age and, most remarkably, maternal dietilestilbestrol (DES) use: daughters whose mothers used DES during pregnancy have 40% higher risk (RR 1.4) of breast cancer risk compared to daughters of non-users (Palmer *et al.,* 2006).

OTHER DISEASES AND ENVIRONMENTAL EXPOSURES

As previously discussed, hyperinsulinemia, but not diabetes mellitus, has been linked to breast cancer risk. Regarding thyroid disorders, a higher proportion of patients with goitre and augmented thyroid gland volume are found among breast cancer patients, without any causal relation so far and probably because cancer patients are more closely monitored than non-cancer patients.

Data about multiple exposures and their link to breast cancer risk are available. Active smoking and, to a lesser extent, second hand smoke, present an association with breast cancer risk. Regarding active smokers, the association seems to be depend on the intensity, duration, and time of the exposure, with a crucial role for the cumulative exposure prior to the first birth . Passive smoking, however, has not yet been observed to present this dose-response association (Johnson *et al.,* 2011; Luo *et al.,* 2011).

No causal relation has been clearly established for other substances such as organochlorated compounds, electromagnetic fields or some medications (digoxin, biphosphonates, statin, antibiotics and antiinflamatory drugs, particularly non-steroidal antiinflamatory drugs, which will be thoroughly discussed in the next section).

GENETIC FACTORS

Important genetic aspects have been indicated by both the occurrence of breast cancer in families and bilateral involvement. Clustering of breast cancer within families is said to have been recorded by the ancient Romans but the first serious publications on this subject in medical literature date back to the 18th century, when Le Dran related the experience of a colleague in Avignon who had diagnosed a 19-year old nun with cancer of the right breast. Her grandmother and a maternal grand-uncle had died from breast cancer and she was convinced that her disease had a hereditary component and that "*her blood was corrupted by a cancerous ferment natural to her family*" (McKusick, 1997). In the 19th century the French physician Paul Broca and his contemporary, the London surgeon Sir James Paget, conducted a specific study of the phenomenon and collected a considerable number of illustrative families, including Broca's own wife. Despite their suspicion that a genetic factor was involved in the disease, and despite the fact that Mendel was their contemporary, they lacked the knowledge of the principles of inheritance and could not apply mathematical modeling to distinguish genetic factors among the different components of multifactorial etiology; this remained an enigma until the final decades of the 20th century, with the discovery of BRCA genes, which will be addressed below (Cornejo-Moreno *et al.,* 2014).

Today, it has been consistenly proven that familial clustering adds a relative risk increase: first-degree relatives of affected individuals have between a 1.5-fold and a 3-fold increased risk (McPherson *et al.,* 2000; Chen, 2014), which is also influenced by diagnosis at a younger age or the person's family history. Moreover, within families with breast cancer

aggregation, patients tend to be diagnosed with cancer in other organs, particularly the ovaries. Subsequently, the existence of hereditary genetic factors has been suggested, which would be involved in about 10% of all breast cancer cases.

In order to quantify the contribution of family risk to total individual risk, familial relative risk (FRR) has been used. It is defined as the quotient between the familiar risk of an individual with an affected relative and the general population risk. Regarding breast cancer, FRR varies with the age of both the healthy individual and their affected relative: the younger the healthy individual and the younger their affected relative, the higher the FRR. A woman under 40 years of age whose first-degree relative was diagnosed also under 40 years of age, has 5-fold higher breast cancer risk compared to a woman of her same characteristics except for the affected relative. However, this difference is reduced to a 50% increase when both ages are over 60. Probably, the most useful contribution of this measure in breast cancer is that, when applied to different environmental factors - which could in theory explain part of that familial risk - no differences have been identified, which highly suggests the existence of purely hereditary factors involved in breast cancer development (Mavaddat *et al.,* 2010).

Gene mutations linked to breast cancer can be classified into three categories, according to their penetrance and their frequency in the general population, which behave in an inverse way: high-penetrance mutations are very infrequent but predispose their carrier very strongly for breast cancer; intermediate-penetrance variants are less frequent and they are moderately associated with breast cancer; finally, low-penetrance variants are much more subtly linked to breast cancer but their frequency in the general population is higher (Table 12).

Table 12. Gene mutations and variants by penetrance, RR and FRR

Locus	Author	Adjacent gene	Variant	MAF *	RR	% FRR**	Protein	Function
High-penetrance mutations								
17q21	Antoniou, 2008	BRCA1	-	0.0006	5-45	10	Breast cancer gene 1	DNA repair
13q12.3	Antoniou, 2008	BRCA2	-	0.001	9-21	12	Breast cancer gene 2	DNA repair
17q13.1	Birch, 2001	TP53	-	infrequent	2-10		Tumor protein 53	DNA repair
10q23.3	Nelen, 1996	PTEN	-	infrequent	2-10		Phosphatase and tensin homolog	Tumor suppressor
19p13.3	Jenne, 1998	STK11	-	infrequent	2-10		Serine/threonine kinase 11	Tumor suppressor
16q22.1	Masciari, 2007	CDH1	-	infrequent	2-10		Cadherin-1	Tumor suppressor
Intermediate-penetrance variants								
11q22.3	Renwick, 2006	ATM	-	0.003	2-3		Ataxia teleangiectasia mutated gene	DNA repair
22q12.1	Meijers-Heijboer, 2002	CHEK2	-	0.004	2-3		Check point kinase 2	DNA repair
17q22-q24	Seal, 2006	BRIP1	-	0.001	2-3		*BRCA1*-interacting protein	DNA repair
16p12.1	Rahman, 2007	PALB2	-	infrequent	2-4		Partner and localizer of *BRCA2*	DNA repair
Low-penetrance variants								
10q26	Easton, 2007	FGFR2	rs2981582	0.38	1.26		Fibroblast growth factor receptor type 2	Cell growth/ signaling
19q13.1	Dunning, 2003	TGF-ß1	rs1982073	-	1.21		Transforming growth factor beta type 1	Cell growth/ signaling
16q12	Easton, 2007	TOX3	rs3803662	0.25	1.20		Chromatin protein	Transcription factor?
5q11	Easton, 2007	MAP3K1	rs889312	0.28	1.13		Serine/threonine kinase	Cell growth/ signaling
8q24	Easton, 2007	FAM84B/ c-MYC	rs13281615	0.40	1.08			
11p15	Easton, 2007	LSP1	rs3817198	0.30	1.07		Cytoskeletal protein	F-acting bundling

3p24	Ahmed, 2009	NEK10/ SLC4A7	rs4973768	0.46	1.11			
17q23.2	Ahmed, 2009	COX11	rs6504950	0.27	0.95	8.3		
10p14	Cox, 2007	CASP8 (D302H)	rs1045485	0.13	0.88		Caspase cysteine protease	Apoptosis
2q35	Milne, 2009	TNP1/IGF BP5/IGFB P2/TNS1	rs13387042	0.52	1.12		Unknown	Unknown
1p11.2	Thomas, 2009	NOTCH2/ FCGR1B	rs11249433	0.40	1.14			
14q24.1	Thomas, 2009	RAD51L1	rs999737	0.24	0.84			
5p12	Stacey, 2008	MRPS30/ FGFR10	rs10941679	0.26	1.19			
6q25.1	Zheng, 2009	ESR1	rs2046210	0.35	1.29			

** MAF in European populations, corresponding to the lowest detected frequency of each alele in that population.** Amount of familial relative risk (FRR) attributable to that variant or mutation*

Modified from Breast Cancer Association Consortium, 2007; Mavaddat et al., 2010; and Cornejo-Moreno et al., 2014)

HIGH-PENETRANCE MUTATIONS

High penetrance mutations were found more than 20 years ago, through studies performed in families which presented multiple cancers in the breast and other organs -mainly the ovaries. These mutations are very rare in the general population (≤1/1000), they almost always behave as autosomal dominant and lead to a very high increase in breast cancer risk (RR 2-50 carriers versus non-carriers). Given its enormous importance, special consideration will be given to BRCA1 and BRCA2.

BRCA1

The confirmation of BRCA1 as a breast-related gene was obtained upon identification of its mutations in families with 17q-linked breast and ovary cancer susceptibility. In 1990, Hall and co-authors reported a Lod score of +5.98 associated with the CMM86 locus on chromosome 17q21 in breast cancer family cases of early-onset disease (Hall et al., 1990). Subsequent efforts to improve the accuracy of mapping for the breast cancer locus were made and finally, in April 1993 Easton et al. (Easton et al., 1993) reported their findings from 214 families and found a substantian number of breast cancer families linked to a gene about 20 cM centromeric of CMM86, which was named BRCA1. The mutation rate for BRCA1 was soon calculated, showing an overall frequency of 0.0007, indicating that about 1 woman in 700 can be considered as a heterozygous carrier. In most cases the linkage was stronger for families with early age at onset. However, after finding BRCA1, its involvement in many families with only breast cancer and almost all families with male breast cancer was ruled out, which in turn suggested a second susceptibility gene (Díez-Gilbert et al., 2006; Cornejo-Moreno et al., 2014). The risk of breast cancer for carriers of BRCA1 mutation by age 70 years has been estimated to range from 40 to 87%, and for ovarian cancer from 16 to 68%. The majority of multi-case families with both breast and ovarian cancers are due to inherited BRCA1 mutations (Díez-Gilbert et al., 2006; Cornejo-Moreno et al., 2014)

Regarding molecular features of BRCA1, it is a large gene extending along 100 Kb of genomic DNA, leading to a 7.8 Kb transcript -abundantly expressed in the testis, thymus, breast and ovary- which is translated to a 1863 amino-acid protein that also encodes a 220 kilodalton (kD) nuclear protein with a zinc-binding RING domain at the amino terminus and a conserved acidic carboxyl terminus. It plays a fundamental role in DNA lesion repair, particularly acting on the double-strand DNA and the cell cycle activation points, to secure a

safe progression to mitosis phase. BRCA1 binds to BRCA2, p53, RAD51 and many other proteins involved in cell cycling and DNA damage response (Lee et al., 2002). Exon 11 generates 60% of the protein and it contains nuclear localization signals to indicate its point of action in the cell, and it interacts with Rad51, p53, Rb and c-Myc. The involvement of BRCA1 in response to DNA damage is supported by extensive data, including evidence that BRCA1 is phosphorylated by the mutated ataxia telangiectasia (ATM) and checkpoint kinase 2 (CHK2) proteins in response to DNA damage (Welcsh et al., 2000). Cells without functional BRCA1 do not arrest in G2 after DNA damage and are deficient in transcription-coupled repair of DNA double-stranded breaks, but the specificity of cancer risk, mostly limited tobreast and ovarian cancer, has not been explained. BRCA1 may regulate the G2-M checkpoint by controlling mitotic spindle assembly and thus chromosome segregation. It is also involved in apoptosis, and the effects of its deficiency are cell cycle dependent (Tutt et al., 2002). BRCA1 presents a highly conserved zinc finger region in its N-terminal extreme, which is likely to be involved in protein interactions that could target proteins for degradation. Mice with homozygously deleted exon 11 of BRCA1 demonstrate chromosomal instability with aneuploidy and chromosome rearrangements (Anand et al., 2003). The C-terminal extreme contains a transcriptional activation domain which interacts with brca2 protein, among others.

BRCA2

Search in families without 17q alterations lead to identification of BRCA2 at 13q12-13, proximal to the retinoblastoma gene (Wooster *et al.,* 1994). The gene was cloned for the first time in December 1995 (Wooster *et al.,* 1995). Risks for female breast, male breast, and ovarian cancers carried a penetrance of 87% by age 80. Families that include male breast cancer cases are more often due to BRCA2 mutations (Hedenfalk *et al.,* 2003). The risks for BRCA2 mutation carriers were estimated to be 40–84% for breast cancer and,11–27% for ovarian cancer. For carriers of BRCA2 mutations a lifetime breast cancer risk of 60–85% and a lifetime ovarian cancer risk of 10–20% were cited. Men with germline mutations in BRCA2, unlike those with germline mutations in BRCA1 had an estimated 6% lifetime risk of breast cancer, a 100-fold increase over the overall male population risk (Cornejo-Moreno *et al.,* 2014).

BRCA2 gene contains 11.385 nucleotides and 27 exons, distributed along 70 Kb of genomic DNA, approximately. Its 10-12 Kb transcript is present in breast epithelial cells and placenta, and it leads to synthesis of a 3418-amino-acid protein. *BRCA2* is even larger than

BRCA1, with a 10.3 kb open-reading frame encoding a 384 kD nuclear protein. *BRCA2* bears no obvious homology to any known gene and the protein contains no well-defined functional domains. BRCA2 binds to BRCA1 and to RAD51. Binding to RAD51 allows BRCA2 to reach sites of DNA breaks, indicating involvement of *BRCA2* as well in recombination-mediated repair of double-stranded breaks and the maintenance of chromosome integrity. *BRCA2* mutations are also associated with an increase in colon, prostate, pancreatic, gallbladder, bile duct and stomach cancer as well as malignant melanoma. *BRCA2* mutants are deficient in spindle assembly checkpoints (Shamoo, 2003). Both genes encode proteins involved in double-stranded DNA repair, specifically in homologous recombination, a highly specific method of error-free DNA repair. Both *BRCA1* and *BRCA2* contain BRCT repeats and both interact with RAD51 – this interaction being along most of the BRCA2 protein, but along only 5% of BRCA1 (Friedman *et al.,* 1994). In cells with deficient BRCA1 and BRCA2 proteins, DNA repair proceeds by the more error-prone alternative repair pathways of non-homologous end-joining. Transcriptional regulation may also be a function of *BRCA1* or *BRCA2* by interaction with RNA Pol II and RNA helicase A. (Both proteins are normally nuclear and their mRNAs are preferentially expressed during the late G1–early S phase of the cell cycle.) Punctate foci of BRCA1, BRCA2 and RAD51 may be detected in the nucleus in the S phase, and in meiotic cells they associate with unsynapsed regions of synaptonemal complexes. None of the functions of *BRCA1/2* appears to be specific to breast tissue. The reason for the tissue specificity in cancer susceptibility is unclear, although the obvious involvement of estrogen-target organs makes it likely that gene mutation effects are enhanced by a responsive tissue environment.

The role of BRCA proteins in breast and ovary tumor development is not yet clear, although the most widely accepted theory is its interaction with estrogen-receptor alpha. Intact brca1 protein inhibits ligand-independent transcription of estrogen-receptor alpha. When mutated, BRCA1 gene results in a truncated protein, such inhibition stops, altering hormonal control of epithelial proliferation, both in the breast and in the ovary.

Mutations in *BRCA1* and *BRCA2* have a prevalence of 0.11% in the general population (Cornejo-Moreno *et al.*, 2014). In families with multiple cases of breast cancer, mutation rates of 57% for BRCA1 and 49% for BRCA2 have been estimated, and 84% and 14% respectively in families with multiple breast and ovarian cancer (Chen S and Parmigiani G, 2007). Approximately 2000 different BRCA1 and BRCA2 mutations have been identified and included in a world-wide data base, constantly under modification (Breast Cancer Information Core, BIC:

http://research.nhgri.nih.gov/bic/). Since these mutations appear with different frequencies in different populations, some geographical areas and especially some ethnic groups present higher breast cancer risk due to such mutations. This is the reason why most mutation lists group their results by country.

The most widely studied group regarding mutations leading to familiar breast is the Ashkenazi Jewish population. Three specific founder mutations have been found and 2% of the individuals are carriers. Three common founder mutations – 187delAG, 5385insC and 6174delT – are present in BRCA1 and BRCA2 in the Ashkenazi Jewish group, which overall occur in up to 2% of this ancestry. The most frequent BRCA1 mutation is 185delAG. It is present in general Jewish population, with a higher prevalence within the Ashkenazi group, and all carriers share a common haplotype. The Ashkenazi founder mutation 6174delT seems to be an example of a low penetrance breast cancer mutation, with estimates as low as 28% (Cornejo-Moreno *et al.*, 2014). The small amount of founder mutations proves the existence of a single ancestor from which all current carriers stem from, and whose mutation was transmitted in the Jewish population through generations. Regarding other countries, certain BRCA2 mutations are also known to be more frequent in Iceland. A common BRCA1 mutation, 2800delAA, is found mainly in Northern Ireland and on the west coast of Scotland, whereas the BRCA2 6503delTT mutation is found on the east coast of Scotland, suggesting that it may have been introduced by Viking raiders or Scandinavian fishermen (Hodgson *et al*, 2004; Genome Bioinformatics Group at Center for Molecular Science and Engineering USCU genome bioinformatics, 2013). Today, 185delAG is one of the most frequent mutations among Spanish population, due to the presence of Jewish groups in the Iberian Peninsula for centuries. Other common mutations in Spain are 243delA (Catalonia) and 330A>G (Gallician) for *BRCA1,* and 3036del4 (found throughout Europe), 6857delAA (Catalonia) y 9254del5 (Mediterranean coast) for *BRCA2* (Díaz-Gilbert *et al.*, 2006).

The involvement of both *BRCA1* and *BRCA2* genes in sporadic ovarian and breast cancer (specifically triple negative tumors) has been suggested, based on the possible hypermethylation of *BRCA1/2* or other genes that act further on the same pathway (Esteller *et al.,* 2000). This hypothesis is especially interesting as far as DNA-repair targeted drugs are concerned, such as platinum-based chemotherapy or PARP inhibitors.

However, cancers in *BRCA1* and *BRCA2* carriers can affect other organs, such as the prostate or the pancreas, which is mostly linked to *BRCA2*. Moreover, biallelic germline BRCA2 and, to a lesser extent, BRCA1 mutations have been found to be associated with Fanconi's anemia, which determines a higher incidence of neoplasia in children. On the other hand, cancers in BRCA1 carriers occasionally present TP53 or PTEN mutations, which are responsible for some of the family syndromes explained in the section below.

OTHER FAMILY SYNDROMES

The remaining high-penetrance mutations have been found in tumor suppression genes and they are linked to family cancer syndromes that induce tumorogenesis in different organs, including the breast.

- Li-Fraumeni syndrome (TP53). Identified in 1990, it is marked by early incidence of sarcoma, leukemia and solid tumors of the adrenal glands, the brain and the breast (Li, 1990).
- Cowden syndrome (PTEN). Also known as hamartomatous tumor syndrome, it presents a higher risk of endometrial, breast and non-medular thyroid cancer. Specifically, it is associated with a 20–30% lifetime risk of breast cancer (Shugart *et al.,* 1999). Up to 75% affected women suffer from benign breast disease.
- Peutz-Jeghers syndrome (STK11/LKB1). It is characterized by hamartomatous polyp formation in the digestive tract and melanin deposits in oral cavity, lips and fingers, with a higher risk of gastro-intestinal, lung, breast, uterus and ovarian cancer. It is associated with a relative risk for breast cancer of 20.3 compared with non-carriers (Boardman *et al.*, 1998).
- Cadherin 1 (CHD1). E-cadherin, mapped on 16q, is a possible candidate for the breast cancer susceptibility gene (Bracke *et al.*, 1994).

Further studies have been able to provide more accurate information about the underlying molecular mechanisms for these mutations to produce breast tumors, but it has been years since the last high-penetrance mutation was identified. Moreover, and despite the high individual increase of cancer risk in their carriers, these mutations only account for 20 to

25% of all familiar cases, due to their low prevalence in general population. Thus, current efforts are focused on the two other types of mutations.

MODERATE-PENETRANCE VARIANTS

The prevalence of moderate-penetrance variants in the general population is slightly higher (1 to 3/1000) and their RR are lower (between 2 and 4). They account for 3 to 5% of FRR. This group comprises a variant that produces a truncated CHEK2 protein, which phosphorylates TP53 and BRCA1; several variants of PALB2 (Rahman *et al.,* 2007) and ATM, involved in DNA repair mechanisms through BRCA2 stabilization and phosphorylation of different proteins, respectively; and BRIP1, which codes a helicase that interacts with BRCA1. A variant in MRE11 has been described, which is part of the MRE11-RAD50-NBS1 complex, tumor suppressor and responsible for genomic stability (Mavaddat *et al.*, 2010). Finally, MSH gene should be mentioned, even if the gynecological cancer most frequently linked to Lynch syndrome is endometrial cancer. However, there is also an increase in ovarian (Bewtra *et al.*, 1992) and probably breast cancer, although the latter hypothesis remains still unconfirmed (Chen, 2014).

- Ataxia-telangiectasia syndrome (*ATM*). This is the only autosomal recessive syndrome in the list. It consists of progressive cerebellar degeneration, cutaneous and ocular vasodilation, immunodeficiency, chromosomic instability, increased sensitivity to ionizing radiation and predisposition to certain cancers, mainly lymphoma and leukemia, but also solid tumors: breast, stomach, ovaries and skin (Suárez *et al*., 2015).
- *CHEK2.* The cell-cycle checkpoint kinase 2 (CHEK2) is an important signal transducer of cellular responses (DNA repair, apoptosis and cell cycle regulation), whose defects have een associated with increased risk for breast cancer. The CHEK2 1100delC mutation has been reported to confer a two-fold increased risk of breast cancer among female carriers and a ten-fold increase in male carriers (CHEK2 Breast Cancer Case-control Consortium CHEK2*1100delC and susceptibility to breast cancer, 2004; Zhang *et al*., 2008; Fletcher *et al*., 2009; Iniesta *et al*., 2010). The frequency of the mutation varies among populations. The highest frequency has been described in Northern and Eastern European countries.
- *BRIP1 (BRCA-1 interacting protein 1).* This gene encodes BRCA-1 interacting protein 1, also known as BACH1 or *BRCA1*-associated C-terminal helicase 1, a DNA helicase which binds to BRCA1 protein, enabling it to develop its functions, which include double-strand break

repair, homologous recombination and transcription activation (Yu *et al.*, 2003; Shiozaki *et al.*, 2004). Two BRIP1 germ line missense mutations have been identified in early-onset breast cancer patients without a family history of breast and ovarian cancer (Cantor *et al.,* 2001; Cantor *et al.,* 2004; Vahteristo *et al.*, 2006).

- *PALB2 (*Partner and localizer of BRCA2). This protein has an important function in the regulation, localization and stabilization of BRCA2. It is involved in the regulation of homologous recombination-based DNA double-strand break repair and in the participation of BRCA2 in the S phase checkpoint (Xia *et al*., 2007; Heikkinen *et al.*, 2009).

LOW-PENETRANCE VARIANTS

Low-penetrance variants have been estimated to explain 75 to 80% of FRR due to their high prevalence in general population (carrier frequency ranges between 13 and 52%, according to the mutation). Although they represent very weak increases of risk when considered individually (RR ≤ 1.2), combinations of several low-penetrance variants can occur, resulting in significant risk increases. In total, more than 90 variants have been found to be involved to date (Fachal and Dunning, 2015).

Most low-penetrance variants are single nucleotide polymorphisms (SNP), which have been identified using two different strategies. First of all, candidate gen method has been used, rendering about 100 genes involved in already known cancer metabolic pathways. Most results obtained with this technique have not yet been replicated, except for one: allele H of CASP8 locus. The second strategy is the use of genomic-wide association studies (GWAS). GWAS consist of simultaneous analysis of thousands of SNPs in very large populations, in order to establish hypotheses based on the differences found. Unlike the candidate gene method, GWAS have been able to demonstrate the association between several loci with breast cancer. Some of these associations have been identified in genes which were already known to be related to breast cancer (FGFR2, TOX3, MAP3K1, LSP1, RAD51L1, PTHLH) and others have been found in regions not containing any known genes (8q24, 2q35, 12q24, 21q21). The actions of these loci -mostly unknown, except for FGFR2- are listed below (Jingmei *et al.,* 2010; Mavaddat *et al.,* 2010; Menashe *et al.,* 2010; Ghoussaini *et al.,* 2012):

- *FGFR2* gene is located in chromosome 10 and it encodes a FRFR2, a receptor for fibroblast growth factor, also known as CD332. It consists of a transmembrane protein, with three immunoglobulin domains in its extracellular region, which bind to fibroblast growth

factors, and a tyrosine kinase domain in its intracellular region. Upon interaction, a cascade of downstream signals is activated, leading to mitogenesis and differentiation. It has been found to be amplified or overexpressed in as much as 10% of human breats tumors, and a SNP in intron 2 of the gene has been found to be associated with a slightly higher breast cancer risk (Hunter *et al.,* 2007).

- *TGF-ß1* is a potent growth suppressor of breast epithelial and carcinoma cells, acting as a negative autocrine growth inhibitor (Artega *et al.*, 1996; Reiss and Barcelloshoff, 1997). However, it is also involved in metastasis formation, and its pro-oncogenic role in advanced disease has been proven (Akhurst and Derynck, 2001; Wakefield and Roberts, 2002). Various polymorphisms of this gene have found, although their association with increased or decreased breast cancer risk has not been clearly identified, except for the Leu^{10}Pro (TGFB1*CC) polymorphism (Kaklamani *et al.*, 2005). Interestingly, some data suggest this SNP increases breast cancer risk by 21% (Dunning *et al.*, 2003), whereas other studies have observed a lower risk (Ziv *et al.*, 2001) or a neutral effect (Krippl *et al.*, 2003; Le Marchand *et al.*, 2004).
- *TOX3/CAGF9/TNRC9K.* This gene encodes the HMG-box nuclear protein TOX high mobility group box family member 3 (TOX3), whose expression pattern and biological functions in the breast are practically unknown (Easton *et al.*, 2007; Stacey *et al.*, 2007). The HMG-box superfamily is defined by a ~80 amino acid DNA-binding domain, and individual members function in regulation of gene expression, chromatin remodeling, genomic stability, DNA repair, and other DNA-dependent cellular processes (Ueda and Yoshida, 2010). Despite the lack of knowledge about TOX3 actions in the breast, its overexpression has been observed in ER+ mammary epithelial cells and in a subset of luminal B breast cancers, with poor outcome (Seksenyan *et al.*, 2015).
- *MAP3K1* gene encodes mitogen-activated protein kinase kinase kinase 1, also known as E3 ubiquitin protein ligase, MEKK, MEKK1, SRXY6, MEKK 1 and MAPKKK1. It consits of a serine/threonine kinase involved in some signal transduction cascades, such as the ERK and JNK kinase and the NF-kappa-B pathway (Hu *et al.,* 2014). A recent meta-analyisis found two SNPs in the MAP3K1 gene which mildly increase breast cancer risk (Zheng *et al.,* 2014).
- *FAM84B/C-MYC*. The importance of these two genes lies in the fact that they are close to the 8q24 locus, which has been identified as a breast cancer susceptibility region, although it does not correspond with any known gene. (Ghoussaini *et al.*, 2008) While C-MYC

overexpression has been largely studied in breast and prostate cancer (Buttyan *et al*., 1987; Nupponen *et al.*, 1998; Sears, 2004), FAM84B encodes a breast cancer membrane-associated protein, with unknown functions (Buttyan *et al*., 1987).

- *LSP1* gene encodes for the lymphocyte-specific protein 1, also known as WP34 and pp52, which is an intracellular F-actin binding protein. It is expressed in lymphocytes, neutrophils, macrophages, and endothelium and it may regulate neutrophil motility, adhesion to fibrinogen matrix proteins, and transendothelial migration. At least two *LSP1*-SNP have been observed to increase breast cancer risk (Easton *et al.,* 2007; Thomas *et al*, 2009; Turnbull *et al*., 2010).
- *NEK10/SCL4A7. NEK10* gene is found on chromosome 3 and it has also been pointed out in different GWAS as a breast cancer susceptibility locus (Ahmed *et al*., 2009; Turnbull *et al.,* 2010; Fletscher *et al*., 2011; Campa *et al*., 2011). It encodes a serine/threonine-protein kinase and its role in breast cancerogenesis remains unclear (Milne *et al*., 2014).
- *STXBP4/COX-11* is the gene which encodes a non-structural protein subunit of cytochrome c-oxidase (COX), the terminal component of the mitochondrial respiratory chain, which catalyzes the electron transfer from reduced cytochrome c to oxygen. COX-11 protein is thought to contain a transmembrane domain localized in the mitochondrial inner membrane. Multiple transcript variants encoding different isoforms have been found for this gene, with differential impact on breast cancer risk: some variants seem to be associated with a lower breast cancer risk (Tang *et al.,* 2012), while others have been observed to increase breast cancer risk (Ahmed *et al*., 2009; Turnbull *et al.*, 2010; Fletcher *et al.*, 2011; Campa *et al.*, 2011).
- *CASP8* is located in crhomosome 2. It encodes a cysteine-protease involved in apoptosis initiation in response to DNA damage. Some of its variants have been linked to a higher breast cancer risk (Cox *et al*., 2007; Turnbull *et al.*, 2010; Campa *et al.*, 2011; Camp *et al*., 2012), whereas others have been observed to decrease it (MacPherson *et al*., 2004).
- *TNP1/IGFBP5/IGFBP2/TNS1*. This gene is found on the 2q35 locus, which has been identified as a breast cancer susceptibility region through various GWAS (Stacey *et al.*, 2007; Milne *et al*., 2009; Thomas *et al.*, 2009; Turnbull *et al*., 2010; Fletcher *et al.,* 2011; Campa *et al.,* 2011). Although no direct association has been identified between the coding IGF binding protein 2 and breast cancer risk, the implication of this pathway in mammary carcinogenesis may support its role.

- *NOTCH2/FCGR1B.* NOTCH proteins are highly conserved transmembrane receptors involved in cell response to hypoxia, stemm cell maturation, migration and inhibition of differentiation (Artavanis-Tsakonas *et al*., 1999; Shimizu *et al.*, 2000; Mumm and Kopan, 2000). This pathway has been largely studied as a potential target for breast cancer (Wu *et al.,* 2007; Hirose *et al*., 2010), which is consistent with the GWAS observations linking some of its variants to an increased breast cancer risk (Thomas *et al.*, 2009; Turnbull *et al*., 2010; Figueroa *et al*., 2011; Campa *et al*, 2011)
- *RAD51L1* is the gene that encodes the DNA repair protein RAD51 homolog 2, also known as R51H2, RAD51B, RAD51 paralog B, REC2 and RAD51L1 (Rapp *et al.,* 1981; Rice *et al.,* 1997; Entrez Gene: RAD51L1 RAD51-like 1 [(S. cerevisiae]). This protein is involved in cell cycle control and DNA repair by homologous recombination and it interacts, among other factors, with BRCA2 (Jensen *et al.*, 2014; Lee *et al.*, 2014). Overexpression of this gene was found to cause cell cycle G1 delay and cell apoptosis, which suggested a role of this protein in sensing DNA damage (West, 2003; Hussain *et al.,* 2004; Sigurdsoon *et al.,* 2001, Miller *et al.,* 2002). Specific SNPs in the RAD51L1 gene might be associated with increased breast cancer risk (Akbari *et al.,* 2010; Meindl *et al.,* 2010; Somyajit *et al.,* 2010; Loveday *et al.,* 2011; Suwaki *et al.,* 2011). Remarkably, a SNP of this gene has been observed to increase breast cancer risk in BRCA-2 mutation carriers (Antoniou *et al.*, 2007), to promote metastasis in patients with triple negative tumors (Wiegmans *et al.,* 2014), to induce trastuzumab resistance (Nam, 2015) and to predict the effect of chemotherapy (Söderlung *et al.,* 2014).
- *MRPS30*. This gene encodes a component of the mithocondrial ribosome, which is essential for oxidative phosphorylation, although its association with breast cancer risk has not yet been elucidated (Quigley *et al.*, 2013). However, it has been identified as a breast cancer susceptibility locus (Stacey *et al*., 2008; Thomas *et al*., 2009; Turnbull *et al*., 2010 Milne *et al*., 2011; Campa *et al*., 2011). Moreover, this locus is close to 5p12 locus is close to FGF10, which is the ligand for FGFR2, whose function has already been explained.
- *ESR1* encodes the estrogen receptor 1, some of whose variants are associated with a higher breast cancer risk, particularly ER+ disease (Zheng *et al.,* 2009; Turnbull *et al*., 2010; Fletscher *et al*., 2011; Campa *et al*., 2011; Long *et al.,* 2012; Son *et al*., 2014). ESR1 regulates estrogen signaling transduction and, when present in high concentrations, it increases breast cancer risk (Nyante *et al*., 2015). Interestingly, there is an increasing

number of studies which correlate ESR1 expression and breast mammographic density (Dumas and Diorio, 2011; Lindström *et al.,* 2014)

- PTHLH encodes PTHrP, the isoform 1 of parathyroid-like hormone, largely expressed in several tissues and tumors, including up to 60% of breast tumors, probably due to its role in apoptosis inhibition and bone metastasis production (Ghoussaini *et al*., 2012).
- 12q24 (Ghoussaini *et al.*, 2012) is close to two genes probably related to cancer: MAPKAPK5 (a protein-kinase that is directly activated by Myc) and TBX3 (involved in breast development and present in high concentrations in ovarian and breast cancer patients, especially ER+). It has been recently discovered how TBX3 is part of the FGF-FGFR-TBX3 metabolic pathway, regulated by estrogen, which favors cancerous stem cell development in the breast. TBX3 is also involved in the Wnt-ß-catenin pathway (Cornejo-Moreno *et al.*, 2014).
- Another susceptibility locus has been found on 21q21. The closest gene is NRIP1/ RIP140. It is a strong nucleus transcription repressor, which inhibits estrogen cell signaling and blocks mitosis (Cornejo-Moreno *et al.,* 2014).
- By 2002, a susceptibility locus in 13q22 gained support for being the hunted *BRCA3* and consensus was found later by the HUGO Gene Nomenclature Committee (HGNC) (Thompson *et al.,* 2002). Nevertheless, genetic testing and information concerning the putative sought-after gene is still scarce and limited.
- The involvement of H-Ras in breast cancer has already been studied. H-Ras expression is associated with human breast epithelial cell invasion and migration, cell proliferation and phenotypic transformation (Yong *et al*., 2011). Individuals with rare alleles of protooncogen HRAS have been observed to present an increased risk of breast cancer (Krontiris *et al*., 1993; Weston *et al.,* 2007; Deb *et al.,* 2014).
- Androgen receptor genes are suspicious susceptibility alleles for male breast cancer (Lobaccaro *et al*., 1993).
- Some polymorphisms calculated from large case-control studies confer small relative risks of breast cancer: V1508M in the *COMT* gene, 1462V in *CYP1A1*, *CYP1B13* (different estrogen metabolism genes). In the human leukocyteantigen (HLA) region, there may be a potential role for the HLA class III sub-region in susceptibility to breast cancer in patients at moderate familial risk (de Jong *et al*., 2003). Regarding *BARD1*, cys557-to-ser substitution in this gene is common and a predisposing factor for allele breast cancer (Karppinen *et al*., 2004). Steroid hormone metabolism genes are associated with a high dominance inherited

breast cancer risk, as is the germline R239X mutation in the *CYP17A1* gene (Hopper *et al*., 2005).

Most SNPs linked to breast cancer are still unknown: the loci identified so far only account for about 8% of FRR, although each week new papers are published describing new mutations and new loci (Michailidou *et al.*, 2015).

LINK BETWEEN LOW-PENETRANCE LOCI AND SPECIFIC TUMOR TYPES

One of the most striking facts about low-penetrance loci is that most of them present a higher association with ER+ breast cancer, as occurs with FGFR2, MAP3K1, 8q24, 5p12, 12q24 and 21q21, whereas TOX3 y 12p11 are equally linked to both ER+ and ER- breast cancer. On the other hand, ESR1 seems to be related to ER- disease (Mulligan *et al.*, 2011). In the case of 12p11, the risk magnitude associated to ER- tumors is one of the highest identified to date (Ghoussaini *et al.*, 2012).

RISK MODULATION IN BRCA1 AND BRCA2 CARRIERS

Not all BRCA1 and BRCA2 mutation carriers have the same breast cancer risk (at age 70, such probability ranges between 40 and 87%) (Isaacs *et al.*, 2012). This interesting disparity is related to the way some environmental factors exert their actions differently according to the type of mutation. For instance, a first birth at age 30 or older is a risk factor for BRCA2 carriers, similarly to the general population, but it behaves as a protective factor for BRCA1 carriers (Friebel *et al.,* 2014). However, since moderate and low-penetrance variants began to be identified, the possibility has been studied that those variants could explain that risk heterogeneity, at least to some extent. In other words, the existence of genetic modifiers of BRCA1 and BRCA2 mutations is being researched.

Concerning this hypothesis, the most interesting finding so far is a SNP in the 5' region of RAD51, a gene that interacts with BRCA1 and BRCA2 by repairing double-strand DNA (Antoniou *et al.*, 2010). Among BRCA2 mutation carriers, those who also present this RAD51 variant have a 3-fold higher risk of breast cancer. However, that variant does not modify breast cancer risk in BRCA1 mutation carriers or in general population (Gaudet *et al.*, 2010).

Other SNPs in FGFR2, MAP3K1, FBXL7, LOC134997 and LSP1 have been shown to cause significantly higher risk increases in BRCA2 than in BRCA1 mutation carriers. Recently

described SNPs in SNRPB and CAMK1D have demonstrated to respectively reduce and increase breast cancer risk in BRCA1 mutation carriers (*Xianshu et al.*, 2010). In the case of TOX2 and 2q35, a similar risk reduction has been found for either BRCA1 or BRCA2 mutation carriers.

When comparing this information with that from the previous paragraph, there seems to exist a parallelism between SNPs linked to BRCA1 and ER- tumors, and between BRCA2 and ER+ tumors. Interestingly, 70% of breast tumors in BRCA1 mutation carriers are ER- and they present a higher frequency of basal-like, while tumors in BRCA2 carriers tend to be ER+, similarly to sporadic tumors.

There are currently three on-going GWAS, one for BRCA1 (HGVST1664) and two for BRCA2 (HGVST656, HGVST160) with the purpose of finding new risk-modifying SNPs for both groups (Hindorff *et al.,* 2014).

PREVALENCE OF LOW-PENETRANCE LOCI IN DIFFERENT POPULATIONS

Since most GWAS have used data from European-ascent populations, there is still little information pertaining to other groups (Mavaddat *et al.*, 2010):

- A SNP in a CASP8 promoter has been shown to increase the risk for multiple cancers, including breast cancer, in a Chinese-origin population, but not in European-ascent populations.
- A variant of ESR1 has been found to cause a higher breast cancer risk increase in Chinese population than in European population and, within the Chinese population, an even higher increase in ER- tumors.
- Besides BRCA1 mutations, a SNP in 6q22 has been linked to breast cancer in Ashkenazi Jewish population.

NEW APPROACHES FOR GWAS RESULTS

GWAS have so far been crucial to find common genetic variants linked to breast cancer. As a consequence, new hypotheses about their molecular mechanisms have been suggested. However, there are important limitations to real application of GWAS results. First of all, GWAS are only suitable for generating hypotheses, which will or will not be confirmed afterwards. Second, the constant finding of new mutations, although scientifically useful, delays the applicability of the already developed hypotheses. Finally, analyses have so far been

performed exclusively SNP by SNP, in such a way that only those reaching a strict level of signification have been considered. The difference between what is statistically significant or not significant, when considering such a large number of polymorphisms, is very small, and thus no attention is paid to thousands of SNPs not included in the results.

The latest trends on breast cancer genetics do not aim so much to start new GWAS as to apply different analysis techniques to draw conclusions from the already published studies. One of the most promising approaches is the SNP grouping by metabolic pathways (pathway-based approach). It consists in estimating whether the cumulative contribution of genes belonging to the same metabolic pathway, regardless of their RRs, is higher than expected by chance. In affirmative cases, it suggests that such pathway could be involved in breast cancer development.

THE COX-2 PATHWAY AND ITS ROLE IN BREAST CARCINOGENESIS

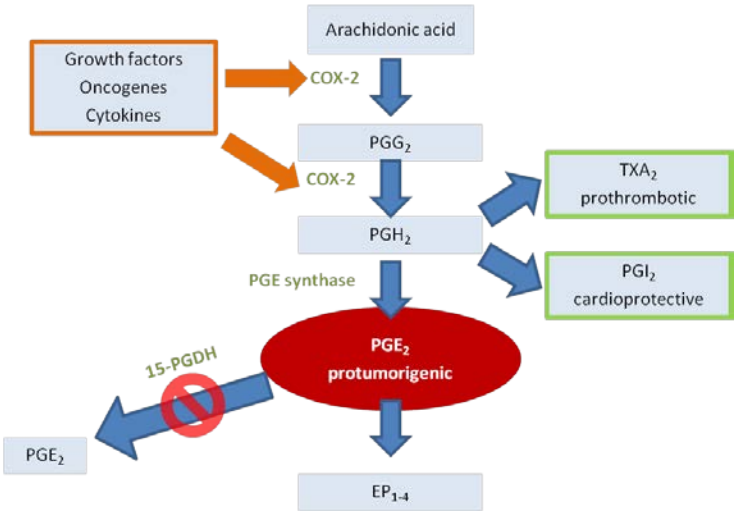
Although aspirin has been comercialized since the end of the nineteenth century, it was not until 1971 that the underlying mechanism for its antiinflammatory effects was discovered (Vane, 1971). That publication suggested that aspirin and all other nonsteroidal antiinflammatory drugs exert their action by inhibiting cyclooxygenase, the rate-limiting enzyme of the prostaglandin cascade. The metabolism of the essential fatty acid, arachidonic acid, *via* the cyclooxygenase pathway results in different prostaglandins that have diverse physiologic actions in the organism, including not only the inflammatory response, but also regulation of constriction of blood vessels, contraction of smooth muscle, aggregation of platelets, sensitization of neurons to pain, flux of intracellular calcium, cell division, apoptosis, and many other molecular events that are critical for homeostatic physiology (Harris *et al*., 2014).

Two primary genes encode cyclooxygenase, a constitutive gene (*COX-1*) and its inducible isoform (*COX-2*) (Williams *et al.,* 1999; Herschmann, 1996). *COX-1* (or, more accurately, *PTGS1* [PG-endoperoxyde-sintase-1]) is expressed ubiquitously, while its inducible form *COX-2* (or *PTGS2* [PG-endoperoxyde-sintase-2]) appears constitutively expressed in only few tissue types (brain, kidney and placenta). However, it can be induced in all other sites upon numerous stimuli, such as cytokines, growth factors and oncogenes, which are in turn secreted in response to all kinds of inflammatory agents: tobacco, alcohol, ischemia, trauma,

pressure, foreign bodies, toxins, bacteria, viruses or lipopolysaccharides (Harris *et al*., 2014). As a consequence of their differential inhibition of COX isoforms, non-steroidal anti-inflammatory drugs (NSAIDs) can be classified into different groups (Table 13).

The cyclooxygenase pathway produces various prostaglandins, prostacyclins and thromboxanes from arachidonic acid and other fatty acids. These substances, also known as prostanoids, contribute to several organic functions: hemostasis and platelet aggregation, gastric and renal function and some female reproductive processes (Williams *et al.,* 1999; Herschmann, 1996). They are also key modulators of inflammation, fever and pain. In the initial step, both COX-1 and COX-2 catalyze the oxidation of arachidonic acid to prostaglandin G_2 (PGG_2) and, subsequently, to prostaglandin H_2 (PGH_2)which is rapidly used as a substrate for multiple isomerases, each of which responsible for synthesis of eicosanoid products such as prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), and tromboxane A_2 (Figure 3). Prostaglandin structure and function depend upon the cell of origin and the level and type of catalytic COX enzyme. *COX-1* is constitutively expressed at basal levels in many cells throughout the body (gastrointestinal epithelium, renal tubules, vascular smooth muscle and blood platelets), maintaining low levels of cytoprotective prostaglandins. Conversely, the *COX-2* gene remains untranscribed unless induced by inflammatory stimuli. Induced COX-2 transcription and expression markedly amplify the biosynthesis of PGE_2, which is the chief effector molecule of inflammation (Clària, 2003) by quickly triggering the biosynthesis and release of PGE_2 (Harris *et al*., 2014).

Figure 3. Eicosanoid metabolic pathway



Cyclooxigense enzimes convert arachidonic acid to the prostanoid halfproduct PGG_2, which, in turn, is converted to PGH_2. The next reactions lead to formation of a group of eicosanoids: Tromboxane A_2 and prostacyclin (PGI_2), products from platelet COX-1 and endothelium COX-2 respectively, seem to play opposite roles in cardiovascular biology. The main molecule in the context of epithelial tumorogenesis, PGE_2, is formed from s PGH_2 through PGE-syntase action. A signal cascade originates from PGE_2 and its interaction with related receptors EP_1 to EP_4. This pathway can be suppressed by 15-hydroxiprostaglandin dehydrogenase (15-PGDH). High PGE_2 levels in neoplastic tissues could, therefore, result either from COX-2 overexpression, or PGE-syntase modulation or loss of expression of 15-PGDH.

Modified from Howe et al., 2007

Table 13. Classification of NSAIDs according to the strengh of COX-1 and COX-2 inhibition

Predominant COX-1 inhibitors (COX-1 > COX-2)	Aspirin	Flurbiprofen	Meclofenamate	Piroxicam
	Diclofenac	Ibuprofen	Mefenamic acid	Sulindac
	Etodolac	Indomethacin	Naproxen	Tiaprofenic acid
	Fenoprofen	Ketoprofen	Oxaprozin	Tolmetin
	Floctafenine	Ketorolac		
Poor COX-1 inhibitors	Acetaminophen		Salsalate	
	Choline magnesium trisalicylate		Bismuth subsalycilate	
	Diflusinal		Bismuth salicylate	
Predominant COX-2 inhibitors (COX-2 > COX-1)	Meloxicam			
	Nabumetone			
	Nimesulide			
Selective COX-2 inhibitors	Celecoxib			
	Etoricoxib			
	Parecoxib			
	Lumiracoxib			

From Stevenson, 2004

Under normal conditions, acute inflammation is a tightly controlled self-limiting response to the offending stimulus, which involves multiple cell types of the vascular and immune systems. During acute inflammation, *COX-2* expression and PGE2 production by endothelial cells, epithelial cells, stromal cells, monocytes and lymphocytes increases up to 100 fold of basal levels (Harris *et al*., 2014). Amplification of the COX-2 inflammatory cascade is triggered by recognition of proinflammatory stimuli by toll-like receptors on the cell membranes of exposed cells and activation of nuclear factor kappa β (NF-κβ) (Lawrence, 2009). In addition, a variety of cytokines (particularly TNFα, γ-interferon, TNF, IL-1 and IL-6) are secreted by infiltrating macrophages and other cells of the innate immune system. These substances trigger the production of acute phase proteins such as C-reactive protein, Amyloid A and complement, which assist in the inflammatory response (Gabay, 2006). Upon abatement of the inflammatory stimulus, IL-1 and IL-6 exert feedback inhibition, which causes COX-2 expression and PGE2 production to cease and the inflammatory process to subside. However, with sustained exposure to pro-inflammatory stimuli, continued overexpression of the COX-2 inflammatory cascade promotes the transition from acute to chronic inflammation (Harris *et al*., 2014). Molecular studies suggest that specific cytokines such as IL-6 and IL-1β are responsible for recruiting monocytes to chronically inflamed tissues which may in turn disrupt the inhibitory feedback loop by secreting a variety of other pro-inflammatory cytokines (Maihöfner *et al*., 2003; Zhao *et al*., 2009).

PROSTAGLANDINS AND CANCER

More than a century ago, Virchow suggested that chronic inflammation leads to cancer development by increasing cellular proliferation (Virchow, 1858; Virchow, 1863; Balkwill and Mantovani, 2001). Different mechanisms of carcinogenesis have been proposed involving inflammatory stimuli and mediators of wound healing (Schreiber and Rowley, 1999; Coussens and Werb, 2002; Philip *et al*., 2004). The discovery of the COX-2 gene has renewed the interest in the association between inflammation and cancer, and various models of carcinogenesis have been suggested involving the specific expression of *COX-2* (Koki *et al*., 2002; Jang and Hla, 2002; Harris, 2002; Harris, 2007).

The role of *COX-2* and its prostanoid products in carcinogenesis has been demonstrated in animal models (Howe *et al.,* 2005; Karmali *et al.,* 1984; Kort *et al.,* 1987; Liu *et al.,* 2001), with effects that include direct mutagenesis, tumor promotion, immunosuppression

(Lupulescu, 1978; Mellemkjaer, 1996), inhibition of apoptosis, angiogenesis and metastasis formation (Harris *et al.,* 2005; Shiff *et al.,* 1999) (Table 14). Transgenic *COX-2* overexpression leads to mammary tumor formation, whereas Cox-2 gene suppression reduces gastro-intestinal, breast and skin tumorogenesis in murine models (Howe, 2007). Consistently with genetic studies, the efficacy of selective COX-2 inhibitors in tumorogenesis suppression has been proved experimentally (Nakatsugi, 2000; Howe, 2000; Howe, 2001; Kubatka, 2003; Howe, 2005).

Aberrant activation of COX/PG pathway in human neoplasia, now well documented, was first researched after finding high PG levels in tumor samples (Williams *et al.,* 1999; Dannenberg *et al.,* 2001). Afterwards, it was suggested that this PG increase and its link with cancer was due to COX-2 overexpression, which is particularly remarkable in colorectal tumors. In fact, COX-2 protein is virtually undetectable in healthy colon mucosa, while more than 85% colorectal adenocarcinomas present high COX-2 protein levels (Williams *et al.,* 1999; Brown *et al.,* 2005). The inverse association between NSAID use and colorectal cancer risk is consistent with this finding (Kune *et al.,*1988; Rosenberg *et al.,* 1991; Rosenber *et al.,* 1998; Thun *et al.,* 1991; Thun *et al.,* 1993; Suh *et al.,* 1993; Muscat *et al.,* 1994; Peleg *et al.,* 1994; Schreinemachers y Everson, 1994; Giovannucci *et al.,* 1995; Smalley *et al.,* 1999; Baron y Sandler, 2000; Coogan *et al.,* 2000; Langman *et al.,* 2000; García-Rodríguez y Huerta-Álvarez, 2001; Thun *et al.,* 2002). Several recent randomized clinical trials have demonstrated how COX-2 inhibitors significantly decrease colorectal adenoma incidence in humans. Unfortunately, those same trials detected an increase in cardiovascular risk associated with the use of COX-2-inhibitors, which suggests an insufficient safety of these drugs for chemoprevention in the general population. Despite this downside, the importance of the COX/PG pathway as an anticancer target has been proved. Regarding cancers in other organs, relatively few studies have been published assessing their association with NSAID use, although a reduction has been found in esophagus and stomach neoplasia (Thun *et al.,* 1993; Funkhouser and Sharp, 1995; Garidou *et al.. 1*996; Farrow *et al.*, 1998, Coogan *et al.,* 2000). However, one study identified a higher pancreatic and prostate cancer risk among NSAID users (Langman *et al.,* 2000). Data pertaining lung and ovarian cancer are contradicting (Peto *et al.*, 1988; Paganini-Hill *et al.*, 1989; Schreinemachers y Everson, 1994; Egan *et al.,* 1996; Harris *et al.,* 1996 and 1999; Cramer *et al.,* 1998; Akhmedkhanov *et al.*, 2001; Moysich *et al.,* 2001; Meier *et al.* , 2002). Most information available is restricted to colorectal cancer. The scarce data on other cancers are often inconclusive, especially considering the necessary dose

or duration for risk reduction (Giovannucci *et al.,* 1995; Rosenberg *et al.,* 1998; Stürmer *et al.*, 1998; García-Rodríguez y Huerta-Álvarez, 2001).

Table 14. Possible mechanisms for NSAID antineoplastic effects

COX-dependent	COX-independent	Unclear dependence from COX
Decreased cell turnover and proliferation	Decreased cell turnover and proliferation	Increased tumor immunity
Increased apoptosis	Increased apoptosis	Induction of myc transcription
Carcinogenesis inhibition	Inhibition of cell transformation	PPAR activation
Angiogenesis inhibition	Increased DNA repair	
	Angiogenesis inhibition	
	Decreased Ras-mediated signal transduction	
	MAP-kinase activation	
	NFκ inhibition	

From Shiff and Rigas , 1999

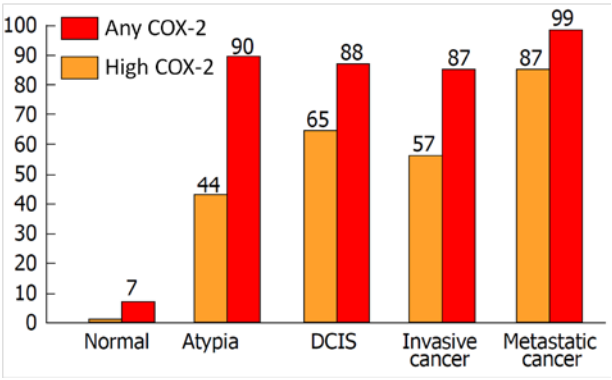
COX-2 OVEREXPRESSION, PROSTAGLANDINS AND BREAST CANCER

Consistently with the findings in other sites, constitutive expression of the *COX-2* gene and sustained biosynthesis of PGE2 seem to be associated to the initiation and promotion of breast carcinogenesis (Harris *et al*., 2014).

The first investigation of *COX-2* in human breast cancer specimens was conducted using immunohistochemistry and a human *COX-2* primer (Parrett *et al*., 1997). The study revealed the presence of COX-2 protein in 13 of 13 invasive human breast tumors, but not in samples of normal breast tissue, with a statistically significant linear association between COX-2 and high (> 50%) tumor cell density ($P < 0.01$) with COX-2 protein localized in tumor cells. Subsequently, multiple independent laboratories have consistently observed *COX-2* overexpression in all stages of breast cancer, ranging from 17% to 84% across different studies (Parret *et al*., 1997; Hwang *et al*., 1998; Soslow *et al*., 2000; Ristimäki *et al*., 2002; Costa *et al*., 2002; Half *et al.,* 2002; Kirkpatrick *et al*., 2002; Davies *et al*., 2003; Kelly *et al.,* 2003; Lim *et al.,*

2003; Spizzo *et al.,* 2003; Watanabe, 2003; Denkert *et al*., 2003; Shim JY *et al*, 2003; Shim V *et al*., 2003; Shingh-Ranger *et al*., 2003; Boland *et al*., 2004; Tan *et al*., 2004; Nakopoulou *et al*., 2005; Perrone *et al*, 2005; Takeshita *et al*., 2005; Barnes *et al*., 2006; Gunnarsson *et al*., 2006; Mehrotra *et al*, 2006; Chuah *et al*, 2011). A review in 2014 found that 87% of specimens were positive for *COX-2* and 57% had high levels of *COX-2* expression, among studies of invasive breast cancer. However, significant high frequencies of specimens with high *COX-2* expression were also observed in premalignant lesions such as atypical hyperplasia (44%) and ductal carcinoma *in situ* (DCIS) (65%) (Harris *et al*., 2014) (Figure 4).

Figure 4. Mean frequency of tissue COX-2 overexpression during progression of breast carcinogenesis

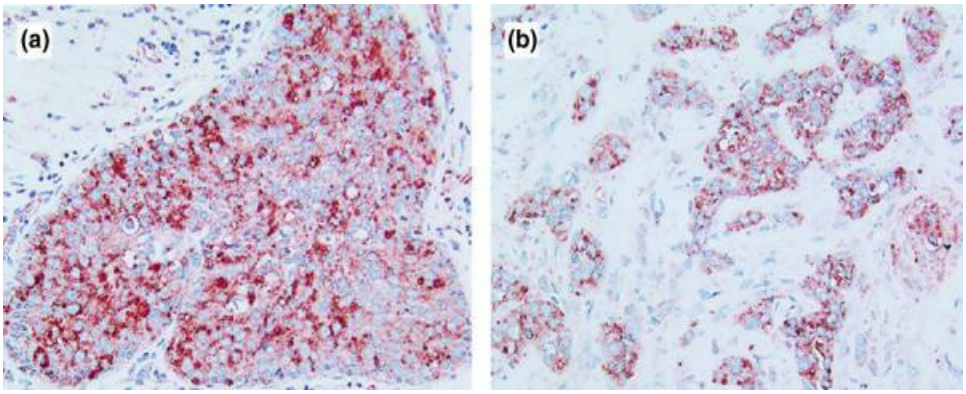


From Harris et al., 2014

In an interesting prospective study, *COX-2* expression was measured by immunohistochemistry in biopsy specimens from 235 women with atypical breast hyperplasia, 17% of whom subsequently developed breast cancer during a median followup of 15 years (Hartmann *et al*., 2006). Notably, *COX-2* expression at baseline was a significant predictor of breast cancer risk. Compared to women without atypia, the cumulative incidence of breast cancer increased with increasing *COX-2* expression (RR 2.6 for weak expression, RR 3.6 for moderate expression and RR 5.7 for strong expression). The authors concluded that "COX-2 *appears to be a biomarker that further stratifies breast cancer risk among women with atypia and may be a relevant target for chemoprevention strategies*" (Hartmann *et al*., 2006; Visscher *et al*., 2008). In the light of this strong molecular evidence, not only does *COX-2* overexpression

constitute an early event in breast carcinogenesis, but it is involved in its whole progression, which makes *COX-2* a potential cancer biomarker and a key target for breast cancer prevention and treatment (Howe, 2001) (Figure 5).

Figure 5. COX-2 expression in human breast tumors

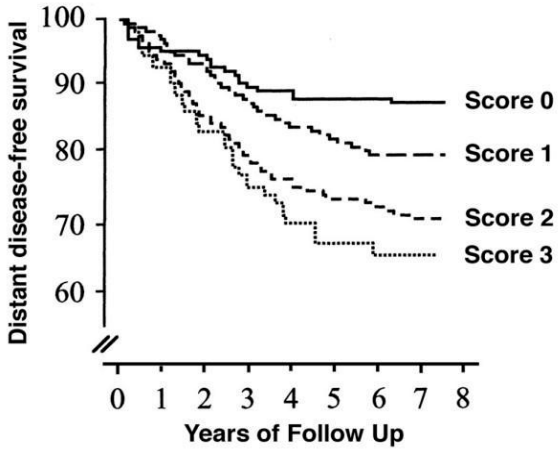


*Cyclooxygenase (COX)-2 protein has been detected in human breast biopsies in both **(a)** ductal carcinoma in situ and **(b)** infiltrating mammary carcinoma using immunohistochemistry on formalin-fixed tissue sections.*

From Howe et al, 2007

A substantial amount of studies (Ristimäki *et al.,* 2002; Subbaramaiah *et al.,* 2002; Denkert *et al*., 2003; Shim *et al*., 2003; Wulfing *et al.,* 2003; Boland *et al.,* 2004; Tan *et al*., 2004; Perrone *et al*., 2005; Takeshita *et al*., 2005; Barnes *et al*., 2006) support that *COX-2* expression is a potential prognostic indicator of disease severity and progression. In contrast, while *COX-1* is expressed ubiquitously in breast tissue (Soslow *et al.,* 2000; Yoshimura *et al.,* 2003), most of those studies have found indetectable or very weak *COX-2* expression in normal tissues. Consistently with these findings, Ristimaky *et al.* identified an inverse association between *COX-2* levels and disease-free period (Figure 6). Induction of *HER2 in vitro* transcription suggests that such relationship is probably causal (Boland *et al.,* 2004; Wulfing *et al.,* 2003; Ritsimaky *et al.,* 2002; Subbaramaiah *et al.,* 2002; Vadlamudi *et al.,* 1999). Interestingly, both *HER2* and *COX-2* are expressed more frequently in *in situ* carcinomas (DCIS) than invasive carcinomas (50-88% and 63-87%, respectively), again suggesting their probable interaction (Boland, 2004; Half, 2002; Shim JY, 2003; Watanabe, 2003; Shim V, 2003; Tan, 2004; Harris *et al*., 2014).

Figure 6. COX-2 expression in breast cancer is linked to lower disease-free survival



Breast cancer patients were classified according to COX-2 expression: score 0 = COX-2 absent (n = 133); score 1 = weak (n = 854); score 2 = intermediate (n = 511); y score 3 = strong (n = 78). High COX-2 protein expression correlated to decreased survival (P < 0.0001; log rank test).

From Ristimaki et al., 2002

EXPERIMENTAL EVIDENCE ON THE ROLE OF COX-2 IN BREAST CANCER

COX INHIBITORS SUPPRESS CANCER IN THE EXPERIMENTAL SETTING

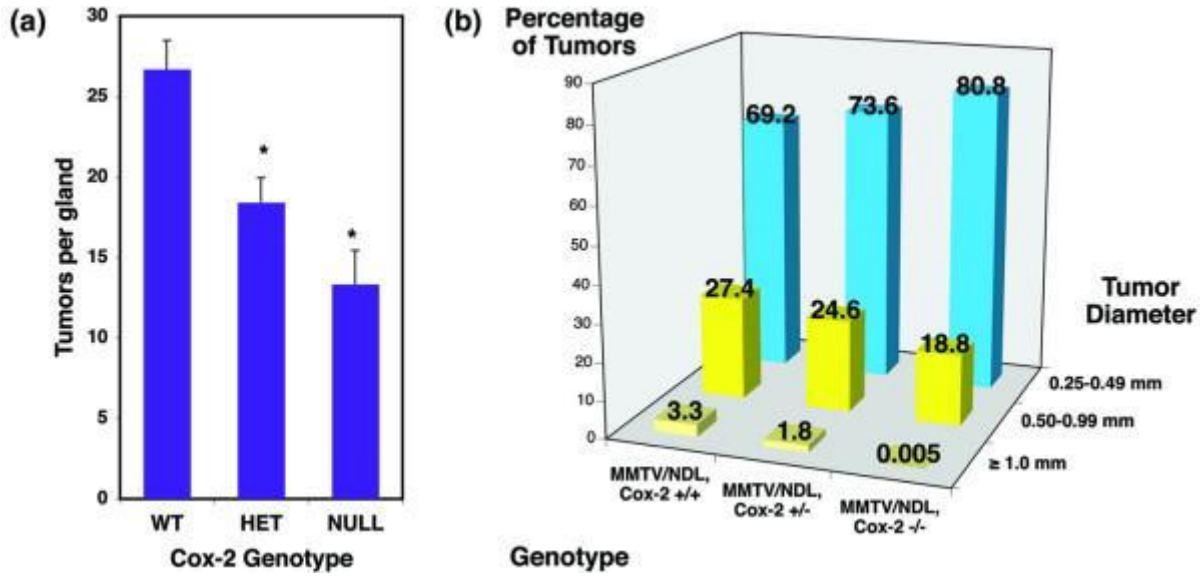
Some human breast tumors cause in vitro osteolysis that may be inhibited by aspirin (Powles *et al.,* 1973). The aspirin metabolite salicylate inhibits breast cancer cells growth and synthesis of the osteolytic IL-6 and IL-11 (Sotiriou *et al.,* 1999). Salicylate also inhibits DNA adduct formation in breast cancer cells (Abbadessa *et al.,* 2006). Aspirin inhibits camptothecin-induced p21CIP1 levels and potentiates apoptosis in human breast cancer cells (Alfonso *et al.,* 2009). Ingestion of aspirin by breast cancer patients has been reported to restore the systemic synthesis of mammary serine protease inhibitor (maspin) through stimulation of systemic nitric oxide production (Bhattacharyya *et al.,* 2010).

The efficacy of COX inhibitors as anticancer agents has been demonstrated in multiple animal models (particularly exhaustive reviews have been published by Howe in 2001 and 2005, Reddy in 2004 and Corpet and Pierre in 2003). The ability of traditional NSAIDs to inhibit breast tumor formation was discovered more than 20 years ago (Karmali *et al*, 1984; Karmali,

2002). These studies also described the differential effects of essential dietary fatty acids in prostaglandin biosynthesis and tumor promotion and showed that supplementation with linoleic acid promoted tumor growth and development by increasing the arachidonic acid metabolism and the PG levels, whereas linolenic acid had the opposite effect.

Similar results were obtained afterwards, using a genetic perspective to describe the involvement of COX-2 in breast tumorogenesis. After a model of *COX-2* gen disruption in an intestinal cancer (Oshima *et al.,* 1996), Howe *et al.* adopted a similar model, crossing *COX-2* knockout mice with *HER2* transgenic mice infected by a mutant strain (NDL) of the murine mammary tumor virus MMTV (MMTV/NDL), to prove the effect of *COX-2* in breast cancer (Howe *et al.,* 2005). MMTV/NDL mice express transgenic HER2, which is activated by a mutation, leading to multiple breast tumor formation, similar to DCIS. Chemically induced tumors tend to be hormone-dependent, which provides a valid model for human breast cancer, given that approximately 2 out of 3 cases are estrogen-positive. These tumors progress to invasive carcinomas and metastasize in the lung, imitating the natural course of the disease in humans (Siegel *et al.,* 1999). Subsequently, MMTV/NDL group was established as a breast cancer model in which the consequences of *COX-2* inactivation could be examined. MMTV/NDL mice were crossed with *COX-2* deficient mice, and tumor multiplicity in *HER2* transgenic mice and *COX-2*-native, -heterozygous and *COX-2*-null mice was compared. Lower tumor multiplicity was identified in both heterozygous and null mice, as compared to *COX-2* native mice ($P < 0.001$) (Figure 7a). Total *COX-2* ablation decreased average tumor multiplicity by 50% approximately. Additionally, a general trend to smaller tumor formation was observed in *COX-2*-null mice as compared to native mice ($P = 0.02$) (Figure 7b), which suggests that not only does *COX-2* contribute to tumor formation but also promotes tumor growth. PGE_2 concentration in MMTV/NDL mice mammary glands correlates with *COX-2* genetic profile: PGE_2 levels (ng/mg protein) were 0.69 ± 0.11 ($n = 7$) for the native form, 0.53 ± 0.15 ($n = 5$; $P = 0.043$) for the heterozygous form, y 0.35 ± 0.07 ($n = 5$; $P = 0.0001$) for the null form. These data provide the first genetic proof of *COX-2* involvement in *HER2*-induced breast tumorogenesis (Howe *et al.,* 2005). In several other preclinical investigations of chemically induced breast cancer, supplemental administration of general NSAIDs such as aspirin, ibuprofen, piroxicam, sulindac, and others, in the diet or drinking water consistently reduced the growth and progression of breast tumors (Joarder *et al*., 1997; Robertson *et al*., 1998; Abou-Issa *et al*., 2002).

Figure 7. Knocking out *COX-2* reduces mammary tumorogenesis



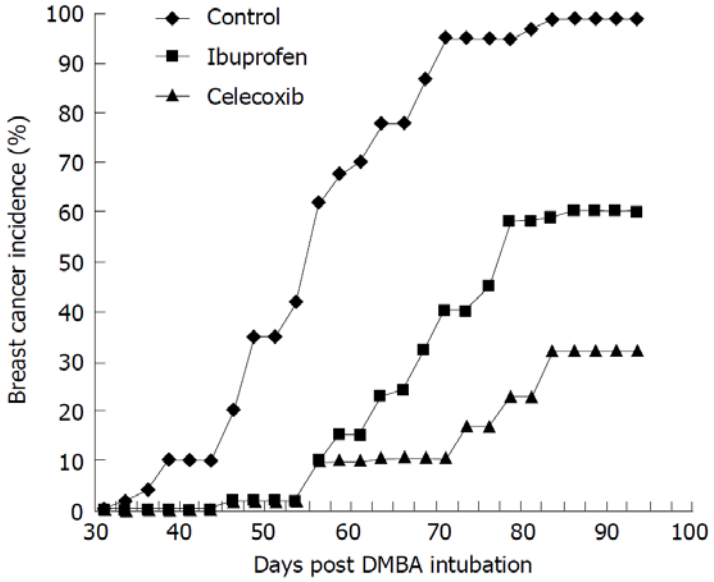
Mouse mammary tumor virus (MMTV)/neu deletion mutant (NDL) mice, which express a mammary-targeted HER2/neu transgene, were crossed with Cox 2-deficient mice, and mammary tumor formation was evaluated in age-matched virgin MMTV/NDL females that were Cox-2 wild type (WT; n = 72), heterozygous (HET; n = 42), and null (NULL; n = 18). ***(a)*** *Tumor multiplicity was significantly reduced in Cox-2 deficient mice (data shown are mean ± SEM. *P < 0.001, by likelihood ratio test.* ***(b)*** *The percentage of tumors in each of the indicated size categories was calculated for each genotype. The proportion of large tumors was significantly reduced in Cox-2 deficient MMTV/NDL animals relative to Cox-2 wild-type controls (P = 0.02).*

From Howe et al., 2005

Afterwards, along with the development of COX-2-inhibitors (celecoxib, rofecoxib, valdecoxib and nimesulide), these drugs were tested in breast cancer animal models, with very strong antineoplastic effects (Nakatsugi *et al.,* 2000; Harris *et al*., 2000; Howe, 2005; Howe *et al.,* 2001, Harris *et al.,* 2000; Kubatka *et al.,* 2003). Harris *et al* (Harris *et al.,* 2000) reported that celecoxib reduced the incidence of breast cancer by 70% and ibuprofen, by 40%, compared to controls in a chemically induced breast cancer model in mice (Figure 8). Further evidence for the primary role of COX-2 in mammary carcinogenesis comes from transgenic mouse models in which the overexpression of COX-2 is sufficient to induce malignant transformation of normal epithelial cells of the mammary gland (Liu *et al*., 2001). That study provided definitive evidence for the oncogenic effect of *COX-2 in vivo*, by creating a

MMTV/*COX-2* murine transgenic strain. *COX-2* overexpression in the mammary gland induced tumor formation in over 85% of multiparous female mice. Before tumors could be visible, COX-2 induced angiogenesis, through increased microvascular density and higher expression of proangiogenic (Chang *et al.,* 2004). On the other hand, breast involution after weaning was delayed in transgenic mice compared to native mice and decreased apoptosis was observed (Liu *et al.,* 2001). According to these data, COX-2 could lead to tumor formation both through angiogenesis and apoptosis suppression. Possible molecular mechanisms involved in *COX-2*-mediated carcinogenesis will be discussed below.

Figure 8. Effects of selective COX-2 blockade on chemically induced breast cancer in mice



From Harris et al., 2014

COX-2 OVEREXPRESSION IN BREAST CANCER

Before discussing the diverse mechanisms through which COX-2 induces carcinogenesis, special attention must be given to the constitutive expression of *COX-2* itself. Multiple molecular studies have shown that breast cancer epithelial cells present an aberrant over-expression of *COX-2* by (Hwang *et al*., 1998; Singh-Ranger *et al.,* 2003; Boland *et al*., 2004; Tan, *et al.,* 2004; Nakopoulou *et al*., 2005; Perrone *et al*., 2005; Takeshita *et al*., 2005). As

previously mentioned, there is abundant evidence on the fact that arachidonic acid production, *COX-2* expression, and prostaglandin biosynthesis are increased *in vivo* by dietary n-6 polyunsaturated fatty acids (n-6-PUFAs) such as linoleic acid, and decreased by n-3-PUFAs such as linolenic acid (Karmali *et al.,* 1985; Rose *et al.,* 1999; Hwang *et al*., 1998; Singh-Ranger *et al.,* 2003; Boland *et al*., 2004; Tan, *et al.,* 2004; Nakopoulou *et al*., 2005; Perrone *et al*., 2005; Takeshita *et al*., 2005). Hence, high dietary intake of n-6-PUFAs may be an important factor in the induction of constitutive *COX-2* expression. This finding is consistent with the high rates of breast, colon and, prostate cancer (which typically overexpress *COX-2*) in populations where dietary intake of n-6-PUFAs is high.

This mechanism is of an utmost importance considering the obesity epidemic in most industrialized nations. The numrerous fat-laden adipocytes in obese patients secrete pro-inflammatory adipokines (*e.g.*, leptin and resistin) and stimulate infiltration by macrophages, which secrete pro-inflammatory cytokines (*e.g.*, IL-6 and TNF-α). Since the *COX-2* gene contains multiple binding sites, these cytokines may also participate in signal transduction cascades to induce constitutive overexpression of *COX-2* and PGE_2 biosynthesis (Shiff *et al*., 1999; Howe *et al*., 2001; Subbaramaiah *et al*., 2003; Subbaramaiah *et al.,* 2011; Subbaramaiah *et al*., 2012; Simpson *et al*., 2013; Rose and Vona-Davis, 2014). Moreover, epigenetic changes in the promoter region of the *COX-2* gene may regulate its own transcription, as observed in *in vitro* studies of breast cancer tissues (Chow *et al.,* 2005).

An important and life-long interaction between adipocytes and mammary epithelium has been demonstrated (Lyon *et al*., 2003). This association has a double function: on the one hand, it provides breast epithelial cells with essential nutrients, which enables morphogenesis, maturation and a correct function of the mammary epithelium; on the other hand, white adipose tissue is an active endocrine organ that secretes a variety of bioactive proteins collectively called adipokines (Nickell and Skelton, 2005).

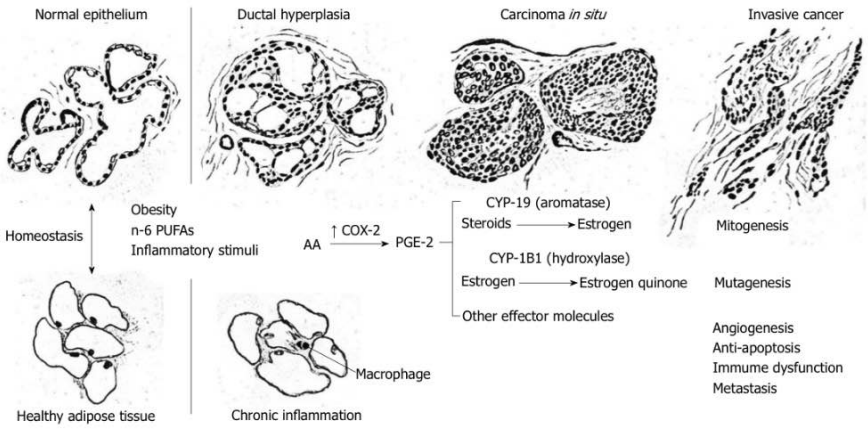
Recent data from the World Health Organization and the International Agency for Research on Cancer suggest a direct effect of the increasing obesity rates on the global rise of breast cancer incidence (20%) and mortality (14%)(Ferlay *et al*., 2013). This finding is consistent with molecular studies in both humans and animals, which show how obesity leads to inflammation and infiltration of mammary and visceral adipose tissue by macrophages, with activation of NF-$_K$B, overexpression of *COX-2,* and hypersecretion of PGE_2 and pro-

inflammatory mediators and adipokines such as leptin, resistin, IL-6, IL-1β and tumor necrosis factors (TNF)-α, as explained below (Subbaramaiah *et al*., 2011; Subbaramaiah *et al;* 2012; Simpson and Brown, 2013; Rose and Vona-Davis, 2014). Moreover, local estrogen biosynthesis, as a consequence of *COX-2* driven PGE_2 biosynthesis and transcription of CYP-19 in the breast parenchyma has been hypothesized to be a key feature of breast cancer development, particularly in postmenopausal women (Zhao *et al*., 1996; Harris *et al.,* 1999), which will be also discussed below.

MOLECULAR MECHANISMS IN COX-2-INDUCED BREAST CARCINOGENESIS

The combination of pharmacological and genetic perspectives has provided solid evidence about *COX-2* contribution to breast cancer. However, a single mechanism explaining such role at a molecular level has not been established. In fact, multiple molecular approaches have been suggested, mainly increased mitogenesis, mutagenesis, and angiogenesis, metastasis formation, inhibition of apoptosis and immunosuppression, which will be further discussed below (Figure 9). However, although anticancer effects of traditional NSAIDs and COX-2-inhibitors seem to definitely involve COX enzimes in breast cancer, numerous COX-independent effects of these drugs have been described (Table 13) (Grosch *et al.,* 2006; Soh and Weinstein, 2003).

Figure 9. Continuous overexpression of COX-2 can initiate and promote carcinogenesis



From Harris et al., 2014

MITOGENESIS

The COX-2 enzyme catalyzes the conversion of essential dietary fats (mainly arachidonic and linoleic acids) into prostaglandins. Overexpression of *COX-2* therefore increases the biosynthesis of PGE_2, which is the key prostaglandin of the inflammatory response. This hormone is capable of inducing the transcription of specific genes that present an important mitogenic action.

AROMATASE

Among them, the aromatase enzyme and its link with COX are currently giving rise to a growing interest. Peripheral aromatization of fatty acids is known to be largely responsible for estrogen production in postmenopausal women, in whom adipose tissue represents an important local source of estrogen. Therefore, regulation of aromatase synthesis in the breast could be particularly important in postmenopausal breast cancer (Zhao *et al.,* 1996; Bulun *et al.,* 2005).

The rise in PGE_2 levels results in the activation of the promoter II region of the aromatase P450 cytochrome gene (*CYP-19*). Aromatase is responsible for estrogen biosynthesis and, therefore, extremely relevant for mammary carcinogenesis, since 60 to 70% breast tumors are hormone-dependent (Zhao *et al.,* 1996). The interesting association, already known, between *COX*, CYP-19 transcription, estrogen biosynthesis and human breast cancer (Brueggemeier *et al.,* 1999; Brodie *et al.,* 2001 Richards *et al.,* 2002; Díaz-Cruz *et al.,* 2006; Subbaramaiah *et al.,* 2011; Subbaramaiah *et al.,* 2012) seems to be causal, since the prostaglandin signaling cascade can stimulate *CYP19* transcription (Díaz-Cruz *et al.,* 2005; Subbaramaiah *et al.,* 2006; Prosperi y Robertson, 2006; Agarwal *et al.,* 1996; Chen *et al.,* 1999; Zhao *et al.,* 1996). This association has also been demonstrated in lung, colon, and prostate cancers, whichs rises the hypothesis that it may constitute a constant feature in cancer promotion and development (Coffey *et al.,* 1997; Mestre *et al.,* 1997; Fiorelli *et al.,* 1999; Pai *et al.,* 2002; Weinberg *et al.,* 2005; Ellem and Risbridger, 2006). CYP19 prostaglandin-dependent induction is reached through cAMP accumulation. At least two isoforms of PGE_2 receptor exist, which work by increasing adenilcyclase activity, with a subsequent transcription of CYP19 from promoters sensitive to cAMP in the surrounding stromal tissue.

Conversely, animal studies have demonstrated a significant reduction in aromatase activity in *COX-2*-null mice, which adds to the hypothesis of the regulating effect of *COX-2* on

aromatase activity in mammary tissue (Agarwal *et al.,* 1996; Zhao *et al.,* 1996; Chen *et al.,* 1999; Narumiya *et al.,* 1999, Muller-Decker *et al.,* 2005; Subbaramaiah *et al.,* 2006; Prosperi and Robertson, 2006).

On the other hand, this mechanism could also explain, at least partially, the decreased breast cancer incidence linked to NSAID use (Friedman and Ury, 1980; Harris *et al.,* 2003; Harris *et al.,* 1996; Khuder and Mutgi, 2001; Schreinemachers y Everson, 1994; Sharpe *et al.,* 2000; Harris *et al.,* 2006; Rahme *et al.,* 2005), since *COX*-inhibition would reduce estrogen concentration in the breast, restricting the growth of estrogen-dependent tumors. It has been suggested that this mechanism could work regardless of the level of expression of *COX-2*, since COX-1 is constitutively expressed in mammary tissue (Soslow *et al.,* 2000; Yoshimura *et al.,* 2003). It has to be remarked that both COX isoforms contribute to tumorogenesis, based on genetic evidence from *COX-1*-null and *COX-2*-null murine strains (Oshima *et al.,* 1996; Howe *et al.,* 2005; Chulada *et al.,* 2000; Tiano *et al.,* 2002). Interestingly, Terry *et al.* (Terry *et al.,* 2004) identified a differential effect of NSAIDs as chemopreventive drugs according to hormone-receptor status. Thus, aspirin use was observed to decrease estrogen-positive breast cancer risk, with no influence on the estrogen-negative disease, which supports the hypothesis that COX-inhibition-mediated breast cancer reduction is due, at least to some extent, to estrogen synthesis suppression.

EGFR

Another possible mitogenic mechanism is PGE_2 activation of *EGFR* that in turn triggers cell division through the mitogen-activated protein kinase (MAPK) cascade (Dannenberg *et al.,* 2005; Bhattacharjee *et al.,* 2010). A study from 1999 discovered that PGE_2 rapidly phosphorylates EGFR and activates the extracellular kinase, ERK-2, thereby stimulating the mitogenic signaling pathway in healthy gastric epithelium and colon cancer (Polakis *et al.,* 1999). Data from that study indicate that PGE_2-induced *EGFR*-transactivation involves signal transduction *via* TGF-α and activated MMP. Other authors have demonstrated this mechanism in precancerous and cancerous tissues of multiple anatomic sites (Henderson *et al.,* 2000; He, 2002; Castellone *et al.,* 2005; Clevers, 2006). In a recent molecular study, *COX-2* expression was detected in cancer cells of more than 95% of specimens and *EGFR* expression was found to be dependent on *COX-2* upregulation (Bocca *et al.,* 2014).

DISRUPTION OF CELL CONTACT INHIBITION

PGE_2 expression seems to be associated with disruption of contact inhibition in malignant cells in specimens of cancerous tissues from multiple anatomic sites (Harris *et al.,* 2014). An accumulation of beta-catenin, a cell adhesion molecule, in the nuclei of cancerous cells, which is controlled by the gene for adenomatous polyposis coli (APC), was first discovered in colon cancer specimens (Polakis, 1999; Henderson, 2000; Whelan and McEntee, 2002; Clevers, 2006). This protein maintains the integrity of a molecular cell adhesion complex, which includes beta-catenin, APC protein, T-cell factor and actin. A series of experiments demonstrated that inhibition of PGE_2 biosynthesis by NSAIDs reduces the accumulation of beta-catenin and the progression of colon cancer (Castellone *et al.,* 2005). Recent molecular studies suggest that this mechanism may be present in other tissues, including the mammary epithelium (He, 2002; Bocca *et al.,* 2014).

MUTAGENESIS

CYTOCHROME P450 SYSTEM

Steady accumulation of DNA damage is believed to contribute substantially to breast cancerogenesis. There is strong experimental evidence to support the role of estrogen metabolites as carcinogenic agents (Maskarinec *et al.,* 2015; Ziegler *et al.,* 2015). Specifically, the metabolism of estrogens by certain enzymes of the cytochrome P450 system (such as aromatase, as previously discussed) result in quinone formation, which damage directly and indirectly DNA. A clear example of this mechanism is found in obese women, with chronically inflamed breast tissue, presenting constantly high levels of PGE_2 and therefore increasing aromatase-driven estrogen biosynthesis. In fact, while most P450 enzyme isoforms are synthesized in the liver, a specific isoform (CYP-1B1) is constitutively expressed in extrahepatic tissues, including the breast. CYP-1B1 preferentially metabolizes estrogen to 4-hydroxyestrogen, which is oxidized to form carcinogenic 3,4 estrogen quinone. This compoung forms unstable adducts with DNA, leading to depurination and mutation *in vitro* and *in vivo*, while reduction of estrogen quinones to hydroquinones and catechols forms reactive oxygen species by redox recycling (Liehr, 1997; Yager, 2000; Cavalieri *et al.,* 2002; Yue *et al.,* 2003; Rogan *et al.,* 2003; Hurh *et al.,* 2004; Xue *et al*., 2005; Yager and Davidson, 2006).

LIPID PEROXIDATION

Lipid peroxidation generates reactive electrophilic compounds that have mutagenic potential. As previously explained, COX catalyzes the oxidation and peroxidation of arachidonic acid to form the intermediate prostaglandins PGG_2 and PGH_2. (Herschman, 1994; Marnett, 1999; Herschman, 2002; Howe *et al.,* 2001; Shiff and Rigas, 1999; Subbaramaiah and Dannenberg, 2003). Spontaneous breakdown of PGH_2 results in the release of malondialdehyde (MDA), which presents a mutagenous effect, although specific enzymes of the cytochrome P450 system and thromboxane synthetase can also catalyze that step (Plastaras *et al.,* 2000). MDA reacts with DNA under normal conditions to form DNA adducts (Marnett *et al.,* 1986). A study demonstrated that induction of *COX-2* in human healthy colon epithelium increased PGE_2, MDA, and characteristic DNA adducts in a similar amount to the levels observed in malignant colon epithelial cells (Sharma *et al.,* 2001). These findings highlight the potential for carcinogenesis due to oxidative damage and mutagenesis attributable to constitutive overexpression of *COX-2* (Harris *et al*., 2014).

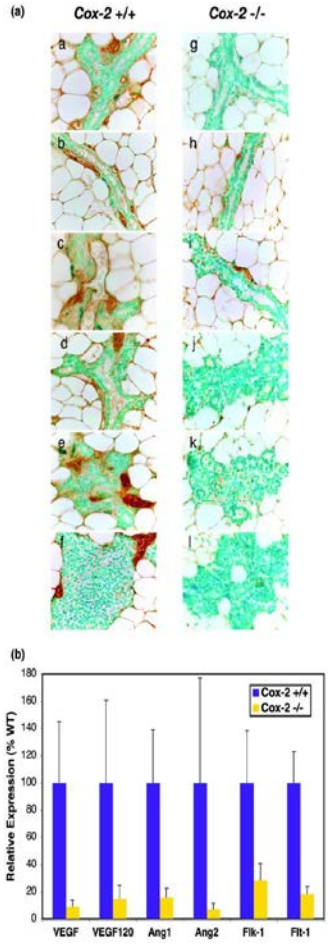
ANGIOGENESIS

VEGF (vascular endothelial growth factor) is a potent stimulant of angiogenesis in a variety of tissues. Once believed present only in the blood vessel endothelium, it has now been discovered in virtually all types of cancers (Masferrer *et al.,* 2000; Folkman, 2006). Molecular studies in breast tumoral tissues provide strong evidence that *COX-2*-derived PGE_2 stimulates the synthesis and release of VEGF resulting in angiogenesis. Newly formed blood vessels are immature and highly permeable, which enables metastatic spread of tumor cells (Koki *et al.,* 2002; Davies *et al.,* 2003; Wang *et al.,* 2007). A positive feedback circuit between tumoral VEGF secretion and amplified *COX-2* expression has been hypothesized to also stimulate lymphangiogenesis (Timoshenko *et al.,* 2006; Zhang *et al*., 2008).

Interestingly, inhibition of this devil's cycle by COX-2 inhibitors has been found to regulate angiogenesis and interrupt the progression and metastatic growth of tumors in animals (Yoshinaka *et al*., 2006). In that study, *COX-2* inactivation also led to the hypothesis that *COX-2* could play a role in mammary gland vascularity. Particularly, a marked reduction in the breast vascular tree was observed in *COX-2*-null mice as compared to *COX-2* -native mice, to the extent that blood vessels were practically absent in both healthy and dysplasic epithelium areas (Figure 10a). Concerning the marked vascularity reduction in mammary tissue

in *COX-2*-null mice, decreased expression of several angiogenesis-related genes was found (Figure 10b), such as *VEGF*, *ANG1* and *ANG2*, *FLK-1* and *FLT-1*. This compares to data from colorectal cancer in mice, which suggest a role for *COX-2* in growth and vascularity of intestinal tumors over 1 mm of diameter (Seno *et al.,* 2002; Takeda *et al.,* 2003). It is therefore possible that *COX-2* contribute not only to tumoral angiogenesis, but also to blood vessel formation in healthy mammary tissue.

Figure 10. Mammary gland vascularization is reduced in Cox-2 knockout mice



(a) Mammary gland tissue sections from age-matched virgin mouse mammary tumor virus (MMTV)/neu deletion mutant (NDL) females that were Cox-2 wild type (subpanels a to f) and Cox-2 null (subpanels g to l) were subjected to anti-CD31 immunohistochemistry, and counterstained with methyl green. Both the number and size of blood vessels were strikingly reduced in Cox-2 null samples.

(b) (b) Expression levels of angiogenesis-related genes were compared by quantitative reverse transcription polymerase chain reaction in MMTV/NDL mammary glands from Cox-2 wild-type (blue columns) and Cox-2 null females (yellow columns). The height of the columns indicates means normalized to the mean expression level of that gene in MMTV/NDL, Cox-2 wild-type samples; the bars indicate the standard error. Expression of VEGF, Ang1, and Flt1 was significantly reduced (P= 0.016, 0.049 and 0.010, respectively). The average of log values across all six genes for each mouse, representing a global effect, was significantly higher in wild-type tissues than in null tissues at P= 0.025.

From Howe et al., 2005

SUPPRESSION OF APOPTOSIS

Apoptosis or controlled cell death is an important regulatory mechanism for the maintenance of homeostasis in cell populations. When this mechanism is altered, it results in immortalization of cells, which is key to cancerogenesis. Inflammation, *COX-2* overexpression, and increased PGE_2 have been demonstrated to present an anti-apoptotic, while COX-2 inhibitors have shown to exert a pro-apoptotic effect (Tsuji and DuBois, 1995; Wu *et al*., 1996; Dubois *et al.,* 1998; Shiff and Rigas, 1999; Subbaramaiah and Dannenberg, 2003).

Apoptosis is regulated both by an intrinsic pathway and an extrinsic pathway, which are inhibited by *COX-2* overexpression (Totzke *et al.,* 2003; Basu *et al.,* 2004; Sugimoto *et al.* 2007). The intrinsic pathway is triggered by a favourable quotient of the nuclear genes BAX and Bcl-2 (i.e., when the quotient favours BAX), with in turn involves mitochondrial production of cytochrome-c and activation of caspase 9 and other lytic enzymes. Notably, *COX-2* overexpression and prostaglandin biosynthesis promotes Bcl-2 and inhibits BAX, blocking the intrinsic apoptosis pathway (Basu *et al.,* 2004; Sugimoto *et al.,* 2007). On the other hand, the extrinsic pathway consists of death receptor activation on the cell surface by TNF-α, TNF-β and other epigenetic factors. This results in activation of caspase 8 and other enzymes that destroy the cell. Similarly to their effect on the intrinsic pathay, overexpression of *COX-2* disables this mechanism thereby blocking extrinsic apoptosis (Totzke *et al.,* 2003).

The observation that COX-2 and PGE_2 inhibitors appear to increase apoptosis, regardless of the pathway, has led to studying their therapeutic features. In fact, COX-2 inhibitors used in combination with radiation have shown beneficial synergism in the elimination of cancer cells in inoperable solid tumors (Petersen *et al.,* 2000; Burg *et al.,* 2002). Nonsteroidal antiinflammatory drugs have also been found to increase apoptosis by other mechanisms, *e.g.*, by increasing bioavailable arachidonic acid pools necessary for conversion of sphingomyelin to ceramide since ceramide accumula- tion in the cell triggers apoptosis (Subbaramaiah *et al.,* 1998) In an interesting study of a breast cell line immortalized by introduction of the human telomerase gene, celecoxib was observed to induce apoptosis and inhibit growth in association with upregulation of IGFBP-3 (i.e.: IGF binding protein-3) (Levitt *et al.,* 2004).

METASTASIS

As explained in the introduction, the *Her-2/Neu* oncogene is a member of the EGFR family and there is evidence of its co-expression with *COX-2* in breast cancer tissues (Koki *et al*., 2002; Subbaramaiah *et al.,* 2002). This co-expression triggers the MAPK/AP-1 signaling cascade, while the sole activation of the Her-2/Neu receptor protein stimulates multiple other factors that promote tumor development and metastatic spread of cancer cells (Koki *et al*., 2002).

Molecular studies of breast cancer tissues have demonstrated that high levels of COX-2 and PGE_2 correlate with amplified *Her-2/Neu* expression and increased activity of matrix metalloproteinases (MMP), a series of proteolytic enzymes that degrade basal membranes, increasing tumor invasiveness, metastastic spread, and poor prognosis (Sivula *et al.,* 2005; Larkins *et al.,* 2006). Consistently, the reduction of Her-2/Neu and MMP levels driven by COX-2 and PGE_2 inhibitors has been demonstrated in animal models of breast cancer, thereby decreasing the metastatic potential of cancer cells (Singh *et al.,* 2005; Kang *et al*., 2011).

IMMUNOSUPPRESSION

Immunosuppression is a characteristic feature of cancer patients that correlates with the course of the disease, including its initiation and progression. As already explained, COX-2 overexpression and high levels of prostaglandins increase cancer cell proliferation, immortalization, and metastasis. However, they also inhibit the function of immune system cells, which results in a patient with a developing tumor, who cannot develop the necessary immune defense to fight it. In fact, it has long been known that the induction of T cell anergy is an early event in the course of tumor progression (Staveley-O'Carroll *et al.,* 1998).

Prostaglandins, and especially PGE_2, are important immunomodulators. A study found that increased levels of PGE_2 suppress the immunocompetence of helper T-cells and dendritic cells in newly diagnosed breast cancer patients (Pockaj *et al.,* 2004). Specifically, elevated levels of PGE_2 were associated with reduced secretion of antitumor factors by T-cells (interferon-gamma, TNF-alpha, and interleukins IL-2 and IL-12) and loss of immunocompetence in dendritic cells (lower secretion of stimulatory molecules, loss of antigen-sensitizing function, reduced phagocytic activity, and lack of maturation potential). Function defects in T- and

dendritic cells due to *COX-2* induced PGE_2 synthesis seems therefore to constitute an important mechanism by which tumors avoid immunosurveillance.

To summarize, the combination of data about *COX-2* expression, animal studies, *in vitro* experiments, and epidemiologic evidence (which will be further discussed in the next section) strongly support the tumorogenic role of *COX-2* in breast cancer and other cancers. The considerable weight of evidence connecting COX/PG pathway, first to intestinal neoplasia, and later to other cancers, including breast cancer, prompted research on NSAID effects as chemopreventive drugs in individuals at risk of cancer. The promising results with traditional NSAIDs have led to similar studies to test the efficacy of COX-2-inhibitors, based on the hypothesis that these drugs would reduce digestive complications linked to traditional NSAIDs. COX-2-inhibitors have been found to decrease incidence of both familiar and sporadic disease, demonstrating the importance of blockage of this pathway (Steinbach *et al.,* 2000; Arber *et al.,* 2006; Baron *et al.,* 2006; Bertagnolli *et al.,* 2006; Grosser *et al.,* 2006). Some studies, however, have shown increased cardiovascular risk related to use of COX-2-inhibitors (Bresalier *et al.,* 2005; Solomon *et al.,* 2005), since PGI_2 leads to a reduction in thrombogenesis, atherogenesis and hypertension and COX-2 inhibition reduces its cardioprotective effect, leaving prothrombotic effects of TXA_2 unaffected. This toxicity has been critical to consider COX-2-inhibitors unsuitable for chemoprevention, but it has been questioned by recent reviews, which have reported their use to be safe if dosage lies within a certain range (Harris *et al*., 2009).

EPIDEMIOLOGICAL EVIDENCE ON COX-2 INHIBITORS AND BREAST CANCER RISK

To this point, abundant and thorough evidence has been provided on the role of the cyclooxygenase/prostaglandin (COX/PG) inflammation pathway in carcinogenesis and on the chemopreventive effect of non-steroidal antiinflammatory drugs (NSAIDs), particularly through the inhibition of the COX-2 isoform (Carter *et al.*, 1983; Hwang *et al.*, 1998; Shiff and Rigas, 1999; Williams *et al.*, 1999; Coussens and Werb, 2002; Thun *et al.*, 2002). The first experimental study demonstrated that NSAIDs block angiogenesis and promote apoptosis in colorectal polyps and further epidemiological studies showed a significant protective effect of NSAIDs against colorectal cancer (Dubé *et al.*, 2007; Rostom *et al.*, 2007). Afterwards, further studies have been conducted in order to explore similar effects of NSAIDs in other neoplasms, including breast cancer, with promising results in the experimental setting. In fact, it has been

proved that both in situ and invasive human breast tumor cells overexpress COX-2 and that COX-2 blockade and overexpression in mice decrease and increase, respectively, breast tumor formation (Harris *et al.*, 2000; Harris *et al*., 2005; Singh-Ranger *et al.*, 2008; Howe and Lippman, 2008).

However, epidemiological studies about NSAID use and breast cancer incidence have not yielded consistent results. Case-control studies (Rahme *et al.*, 2005; Harris *et al.*, 1995; Harris *et al.*, 1995; Rosenberg, 1995; Harris *et al.*, 1996; Neugut *et al.,* 1998; Coogan *et al.*, 1999; Langman *et al.*, 2000; Cotterchio *et al.*, 2001; Meier *et al.*, 2002; Moorman *et al.*, 2003; Terry *et al.*, 2004; Swede *et al.,* 2005; Zhang *et al.*, 2005; Harris *et al.*, 2006; Shen *et al.*, 2006; Vogel *et al.*, 2007; Chan *et al.*, 2007; Davis and Mirick, 2007; Kirsh *et al.*, 2007; Slattery *et al.*, 2007; Brasky *et al.*, 2010; Cronin-Fenton *et al.*, 2010; Ashok *et al.*, 2011, Brasky *et al.*, 2011; Brasky *et al.*, 2011; Vinogradova *et al.*, 2011; Jonsson *et al.*, 2011; Ou *et al.*, 2013) globally support a small decrease in breast cancer risk with NSAID use: a protective effect of NSAIDs was demonstrated in 13 studies (Rahme *et al.*, 2005; Harris *et al.*, 1995; Rosenberg, 1995; Harris *et al.*, 1996; Neugut *et al.*, 1998; Cotterchio *et al.*, 2001; Moorman *et al.*, 2003; Swede *et al.,* 2005; Kirsh *et al.*, 2007; Slattery *et al.*, 2007; Ashok *et al.*, 2011; Ou *et al.*, 2013), while only 7 papers showed a higher risk of breast cancer among antiinflammatory drug users (Harris *et al.*, 1995; Langman *et al.*, 2000; Zhang *et al.*, 2005; Vogel *et al.*, 2007; Davis and Mirick, 2007; Vinogradova *et al.,* 2011; Schreinemachers and Everson, 1994). A neutral result was found in one case-control study (Kirsh *et al.*, 2007) (Table 15). Cohort studies (Friedman and Ury, 1980; Paganini-Hill *et al.*, 1989; Thun *et al.,* 1993; Schreinemachers and Everson, 1994; Egan *et al.,* 1996; Harris *et al.*, 1999; Sharpe *et al.*, 2000; Friis *et al.*, 2002; Johnson *et al.*, 2002; Friis *et al.,* 2003; Harris *et al.*, 2003; Sørensen *et al.*, 2003; Ratnasinghe *et al.*, 2004; García Rodríguez and González-Pérez, 2004; Jacobs *et al.,* 2005; Marshall *et al.*, 2005; Gallicchio *et al.,* 2006; Gallicchio *et al.,* 2007; Gill *et al.*, 2007; Jacobs *et al.,* 2007; Bardia *et al.,* 2007; Friis *et al.*, 2008; Ready *et al.,* 2008; Gierarch *et al.,* 2008; Siemes *et al.*, 2008; Eliassen *et al.,* 2009; Bardia *et al.*, 2011; Bosco *et al.*, 2011; Zhang *et al.*, 2012), on the other hand, show very modest risk differences both as a protective (Paganini-Hill *et al.*, 1989; Schreinemachers and Everson, 1994; Harris *et al.*, 1999; Sharpe *et al.*, 2000; Johnson *et al.*, 2002; Friis *et al.,* 2003; Harris *et al.*, 2003; Sørensen *et al.*, 2003; Ratnasinghe *et al.*, 2004; García Rodríguez and González-Pérez, 2004; Gill *et al.*, 2007; Friis *et al.*, 2008; Ready *et al.,* 2008) and as a risk factor [Egan *et al.,* 1996; Sørensen *et al.*, 2003; Jacobs *et al.*, 2005; Marshall *et al.*, 2005; Jacobs *et al.*, 2007; Friis

et al., 2008; Siemes *et al.*, 2008), with one study showing a neutral OR (Friis *et al.*, 2002) (Table 15).

Data from randomized clinical trials are exceptional: only two papers (Cook *et al.*, 2005; Zhang *et al.*, 2008) were found in the preliminary search and both refer to the same study (Table 16).

To date, 11 meta-analyses regarding NSAID use and breast cancer incidence have been published, but one of them was a Japanese publication and it has not been reviewed here (Asaga *et al.*, 2012). The 11 remaining studies (Khuder and Mutgi, 2001; González-Pérez *et al.,* 2003; Bosetti *et al.*, 2006; Mangiapane *et al.*, 2008; Takkouche *et al.*, 2008; Harris, 2009; Zhao *et al.,* 2009; Algra *et al.*, 2012; Bosetti *et al.*, 2012; Luo *et al.*, 2012;, Tolentino *et al.*, 2012) support a modest protective effect of these drugs (Table 17). There is an evident difficulty in performing those meta-analyses, given the differences among the studies already mentioned; but it is also difficult to compare their results, mainly due to the heterogeneity in both the inclusion criteria and in the drugs assessed in each meta-analysis: 5 of them include different types of NSAIDs (Terry *et al.*, 2004; Harris *et al.*, 2006; Gill *et al.*, 2007; Eliassen *et al.,* 2009; Brasky *et al.*, 2010), the rest consider either only aspirin (Bosetti *et al.*, 2006; Mangiapane *et al.*, 2008; Algra *et al.,* 2012*;* Luo *et al.*, 2012) or non-aspirin NSAIDs (Tolentino *et al.*, 2012).

OBJECTIVES

There is abundant evidence on non-steroidal antiinflammatory drugs (NSAID) use and breast cancer risk, showing a slightly protective effect of these drugs, despite the lack randomized clinical trial results and the high heterogeneity in exposure measurement. However, the most recent meta-analyses have failed to assess two crucial factors: the wide range of NSAID types -especially COX-2 inhibitors- and the way differential expression patterns of homonal receptors and inflammation-related genes modify the effect of NSAIDs on breast cancer incidence. Therefore, we carried out a meta-analysis intended to

1. Update previous meta-analysis on breast cancer – NSAID use with the evidence published from 2009 onwards.

2. Establish the effect of several types of NSAIDs on breast cancers altogether and on estrogen-receptor positive breast cancer, prostagen-receptor positive breast cancer and Her2 positive breast cancer.

3. Identify whether COX-2 inhibitors have a protective role on breast cancer.

METHODS

TYPE OF STUDY

In order to upraise the knowledge about NSAID use and breast cancer risk and solve the aforementioned problems, a systematic review and new meta-analysis were performed. The information from the last meta-analysis was updated, and the focus was set on the evidence on specific effects of COX-2 inhibitors and differential expression patterns of hormonal receptors.

Meta-analyses were performed separately for case–control and cohort studies, for different NSAID types and for different hormone-receptor status. This classification is not only intended to assign a pooled RR/OR to each exposure, but also to suggest further hypotheses for breast carcinogenesis and to clarify the inconsistencies found among the results of previous studies.

SEARCH STRATEGY

A PubMed database search was conducted to include all the entries published with the keywords “BREAST CANCER NSAID ANTIINFLAMMATORY” until October 24th, 2013 resulting in 1508 articles. This initial nonspecific search was chosen in order to cover all relevant publications. Titles and abstracts were evaluated subsequently; articles were selected if they accomplished all of the following inclusion criteria: (a) They report original results from cohort studies, case-control studies or randomized clinical trials; (b) they report at least one relative risk (RR) or odds ratio (OR) of the association between any NSAID use (aspirin and non-aspirin, COX-2-specific and nonspecific) and invasive breast cancer incidence.

Applying these criteria, 49 publications were identified: 23 case-control studies, 24 cohort studies and 2 papers from the same randomized clinical trial. Studies regarding the association between specific polymorphisms in inflammation-related genes and breast cancer according to the use of NSAIDs (Shen *et al.*, 2006; Vogel *et al.*, 2007; Brasky *et al.*, 2011) have been excluded due to the lack of a general RR/OR for NSAID - breast cancer relationship irrespective of genetic features.

DATA EXTRACTION

The following basic information was retrieved in each article when available: (a) Study characteristics: Type of study (controlled clinical trial/cohort study/case-control study), number of subjects at baseline and number of recorded cases. (b) Exposure characteristics: type of NSAID, characteristics of its use (frequency, intensity, duration, and dose). (c) Breast cancer characteristics: type of breast cancer, presence or absence of hormone receptors (for estrogens or progesterone), positivity to Her-2 receptors. (d) Measure of NSAID – breast cancer association: OR/RR with their 95% confidence interval (CI).

STATISTICAL ANALYSIS

The statistical analysis was performed separately for cohort and case-control studies; the unique controlled clinical trial found was included in the cohort study analysis. We carried out separate analysis for any combination of type of NSAID/type of breast cancer reported in at least three studies. According to the type of NSAID, we have considered the analysis of "any type of NSAID", aspirin, non-aspirin NSAID, ibuprofen, acetaminophen, or COX-2 inhibitors. Many studies reported several results for different doses or different durations of treatment with NSAIDs; the ways doses or lengths were reported were not standardized across studies, making it difficult to extract them in an analyzable form. Therefore, in order to magnify the effect of NSAIDs, we selected the OR or RR reported for the highest dose or the longest duration of treatment. According to the type of breast cancer, we contemplated all invasive breast cancers, estrogen positive breast cancers, progesterone positive breast cancers, and receptor-negative breast cancer.

A pooled OR or RR has been estimated weighing individual results by the inverse of their variance (Brockwell and Gordon, 2001); a fixed-effect model was preferred if Q statistics were higher than 0.1, indicating no significant heterogeneity; a random-effect model was chosen otherwise (DerSimonian and Laird, 1986). OR or RR heterogeneity was measured using Q and I^2 statistics (Higgins and Thompson, 2002). Q is an estimator of the homogeneity between studies; it allows to estimate a p-value which would be used for rejecting the null hypothesis of homogeneity; however, it is well known that Q has low statistical power; therefore, the usual threshold for rejected homogeneity is p = 0.1. I^2 indicates the proportion of the effect variability due to heterogeneity between studies.

The presence of small-study bias was explored with Egger test (Egger *et al.*, 1997); due to its low sensitivity, the cut-off was set at p = 0.1. Funnel plots (Light *et al.*, 1984) and the trim and fill method (Duval and Tweedie, 2000) were applied to detect publication bias. In particular, the trim and fill method assumes that the most negative (i.e.: no NSAID effect) studies are missing or suppressed; then, if it detects a bias, it simulates the results of the studies presumably missed (Jin *et al.*, 2015). In such a case, two pooled OR/RR are reported: the one reached with the original data and the one obtained by filling the (presumed) missing studies; this corrected OR/RR should be interpreted as a sensitivity analysis rather than as a true estimator (Jin *et al.*, 2015). Results from theEgger test and trim and fill method are here reported only when relevant.

An analysis of influence was performed via re-estimating pooled OR/RR by removing one study at a time. Studies that, when removed, strongly changed the OR/RR would be considered as highly influential. Results are displayed as forest plots showing OR/RR and their 95% confidence intervals for each individual study and for the pooled result.

Cumulative meta-analyses were carried out in order to know the stability of the OR/RR estimations. In order to do that, all studies considered were arranged from older to newer. Then an OR/RR estimation was obtained for the two eldest studies; another for the three eldest, and so on, adding a study each time. Results are reported as forest plots.

Galbraith radial plots were used for studying heterogeneity. In brief, for each study, the natural logarithm of OR/RR is standardized by dividing it for its standard error. The result is displayed in a scatter plot against its precision (=1/standard error). For any study, the measure of effect would be represented by drawing a straight line between the point representing the study and the origin (i.e.: lnOR/SE=0, 1/SE=0). The angle that this line formes with the horizontal represents the OR/RR. Therefore, studies displaying the same angle have the same OR/RR. Angles over the horizontal indicate OR/RR>1 (i.e.: risk factor), while angles under the horizontal indicate OR/RR<1 (i.e.: preventing factor). Confidence bands are drawed showing the expected random variability. Studies dropped outside the confidence band are considered a possible source of heterogeneity.

All the statistical analyses were carried out with the package Stata 12/SE (Stata Corporation, College Station, TX, US).

RESULTS

NSAID CONSUMPTION AND BREAST CANCER (ALL TYPES)

RELATIONSHIP BETWEEN ANY NSAID AND BREAST CANCER

Twenty-one case-control studies and 12 cohort studies provide results on any NSAID–breast cancer relationship (Tables 14, 15). Analyzing all case-control studies (Rosenberg, 1995; Harris *et al.,* 1995; Harris *et al.,* 1995; Harris *et al.,* 1996; Neugut *et al.,* 1998; Coogan *et al.,* 1999; Langman *et al.,* 2000; Cotterchio *et al.,* 2001; Meier *et al.,* 2002; Moorman *et al.,* 2003; Terry *et al.,* 2004; Rahme *et al.,* 2005; Zhang *et al.,* 2005; Swede *et al.,* 2005; Harris *et al.,* 2006, Kirsh, 2007; Davis and Mirick, 2007; Slattery *et al.,* 2007; Brasky *et al.,* 2010; Cronin-Fenton *et al.,* 2010; Ashok *et al.,* 2011), we obtained a pooled OR of 0.90 (95% CI: 0.88- 0.91) using the fixed effects model, and an OR of 0.82, (95% CI: 0.77-0.88) using the random effects model, which supports a protective role of NSAID consumption against breast cancer (Table 18, Figure 11a). The fixed-effects model shows a high heterogeneity among the results from the different studies (I^2 = 85.9%), which does not differ significantly from previous meta-analyses (Table 17). However, most of the heterogeneity has been eliminated using the random-effects model (I^2 = 38.9%). Although Egger test cannot rule out a small-study effect (p = 0.04), no study shows a relevant influence (Figures 11a, 12a, 13a).

The meta-analysis of cohort studies (Harris *et al.,* 1999; Sharpe *et al.,* 2000; Johnson *et al.,* 2002; Harris *et al.,* 2003; Jacobs *et al.,* 2005; Marshall *et al.,* 2005; Gallicchio *et al.,* 2007; Gill *et al.*, 2007; Friis *et al.,* 2008; Ready *et* al., 2008; Siemes *et al.,* 2008; Bardia *et al.,* 2011) rendered a pooled RR 0.97 (95% CI 0.94-1.00) and, using the fixed effects model and the random effects model, respectively, which shows a non-significant protective effect (Table 18, Figure 11b). There was a high degree of heterogeneity (I^2 = 89.9%), although most of it is eliminated using the random effects model (I^2 = 37.8%). No study shows a relevant influence (Figures 11b, 12b, 13b). Egger test could not exclude the possibility of a small-study bias (p = 0.098). However, when the trim and fill method was applied, results remain virtually unchanged in both case-control and cohort studies, reducing the possibility of small-study and publication biases.

RELATIONSHIP BETWEEN ASPIRIN AND BREAST CANCER

A total of 11 OR provided by 10 case-control studies (Harris *et al.,* 1996; Neugut *et al.,* 1998; García Rodríguez and González-Pérez, 2004; Terry *et al.,* 2004; Swede *et al.,* 2005; Zhang *et al.,* 2005; Harris *et al.,* 2006; Slattery *et al.,* 2007; Cronin-Fenton *et al.,* 2010; Brasky *et al.,* 2011) evaluating aspirin use and breast cancer risk were considered for the meta-analysis, including two estimates (Her2+ and Her2-) from Brasky's study (Brasky *et al.,* 2011), with a pooled OR of 0.87 (95% CI: 0.83-0.92) which points to a protective effect against breast cancer (Figure 15a). No study shows a relevant influence (Figures 15a, 16a, 17a) and Egger test excludes the possibility of a small-study bias (p = 0.13).

Information on aspirin use and breast cancer risk has been found in 11 cohort studies (Paganini-Hill *et al.,* 1989; Schreinemachers and Everson, 1994; Egan *et al.,* 1996; Friis *et al.,* 2003; Ratnasinghe *et al.,* 2004; Marshall *et al.,* 2005; Gallicchio *et al.,* 2007; Gill *et al.,* 2007; Friis *et al.*, 2008; Ready *et al*., 2008; Zhang *et al.,* 2012) and one randomized trial (Zhang *et al.,* 2008), resulting in a non-significant pooled RR of 0.98 (95% CI: 0.95-1.02) (Figure 15b). No study shows a relevant influence (Figures 15b, 16b, 17b), and Egger test excludes the possibility of a small-study bias (p = 0.19).

RELATIONSHIP BETWEEN IBUPROFEN AND BREAST CANCER

We found six case-control studies containing data on ibuprofen use and breast cancer incidence (Harris *et al.,* 1996; Terry *et al.,* 2004; Swede *et al.,* 2005; Zhang *et al.,* 2005; Harris *et al.,* 2006; Brasky *et al.,* 2010) with a pooled OR of 0.87 (95% CI: 0.80-0.94) and 0.83 (95% CI 0.69-1.00), using the fixed effects model and the random effects model, respectively (Figure 18). A moderate heterogeneity was detected (I^2= 72.5%), although most of it has been eliminated usind the random effects model (I^2= 22.0%). Egger test excludes the presence of a small-study bias (p = 0.43) and no study shows a relevant influence (Figures 19-21).

Only one cohort study (Marshall *et al.,* 2005) provides specific data on ibuprofen use and breast cancer risk, showing a non-significant association: RR 1.09 (95% CI: 0.99 – 1.20).

RELATIONSHIP BETWEEN ACETAMINOPHEN AND BREAST CANCER

Information about the use of acetaminophen and breast cancer was provided by 8 case-control studies (Harris *et al.,* 1996; Terry *et al.,* 2004; García Rodríguez and González-

Pérez, 2004; Swede *et al.,* 2005; Zhang *et al.,* 2005; Harris *et al.,* 2006; Brasky *et al.,* 2010; Ashok *et al.,* 2011). The pooled OR calculated for this meta-analysis is 0.90 (95% CI 0.85-0.95) and 0.85 (0.76-0.95), using the fixed effects model and the random effects model, respectively (Figure 22). The heterogeneity among studies was moderate (I^2= 63.2%), although an important decrease was detected using the random effects model (I^2= 8.7%). Small-study bias can be ruled out using Egger test (p = 0.10), although this result lies in the low limit of the cut-off value. However, when the trim and fill method was executed, it ensured the absence of small-study and publication biases. Moreover, the influence analysis did not highlight any study as particularly influential (Figures 23-25).

Data on acetaminophen use and breast cancer have been found in two cohort studies (Friis *et al.,* 2008; Zhang *et al.,* 2012), with a pooled RR of 0.95 (0.88-1.01) with a low heterogeneity (I^2 = 0.75%).

RELATIONSHIP BETWEEN NON-ASPIRIN NSAID AND BREAST CANCER

Only two among all the case-control studies (García Rodríguez and González-Pérez, 2004; Cronin-Fenton *et al.,* 2010) consider the use of non-aspirin NSAIDs as a group, with a pooled OR of 1.02 (0.98-1.07).

There are 8 cohort studies reporting RR on breast cancer incidence and use of non-aspirin NSAIDs (Johnson *et al.,* 2002; Sorensen *et al.,* 2003; Gill *et al.,* 2007; Friis *et al.,* 2008; Ready *et al.,* 2008; Siemes *et al.,* 2008; Bardia *et al.,* 2011; Zhang *et al.,* 2012). The pooled RR is 1.03 (0.99-1.08) and 1.04 (0.98-1.12), using the fixed effects model and the random effects model, respectively (Figure 26). A moderate heterogeneity (I^2 = 43.6%) is found using the fixed effects model, with a lower value in the random effects model (I^2 = 4.36%). Egger test rejects the possibility of small-study bias (p = 0.36). The trim and fill method indicated that two studies had been missed; when added, the corrected RR (random-effect) was 1.02 (0.95-1.09). The influence analysis did not highlight any study (Figures 27-29).

RELATIONSHIP BETWEEN COX-2 INHIBITORS AND BREAST CANCER

Data on the use of COX-2-inhibitors and breast cancer risk has been identified in 6 studies: 5 case-control studies (Rahme *et al.*, 2005; Harris *et al.*, 2006, Cronin-Fenton *et al.*,

2010; Ashok *et al.*, 2011; Vinogradova *et al.*, 2011) and 1 cohort study (Siemes *et al.,* 2008), so only a meta-analysis on case-control studies could be performed. Among the 5 remaining studies, 3 of them provide different ORs for specific COX-2-inhibitors: Rahme *et al.,* 2005 and Harris *et al.,* 2006 provide separated ORs for celecoxib and rofecoxib; while Ashok *et al.,* 2011 provides separate results for celecoxib, rofecoxib and valdecoxib (Rahme *et al.,* 2005; Harris *et al.,* 2006; Ashok *et al.,* 2011); the 2 remaining studies consider COX-2-inhibitors as a group and provide only a pooled OR (Cronin-Fenton *et al.,* 2010; Vinogradova *et al.,* 2011). Therefore, a total of 9 ORs from 5 studies were included in the meta-analysis.

The combined estimate of ORs from these case-control studies in the meta-analysis is 0.90 (0.87-0.93) and 0.85 (0.73-0.98), using the fixed effects model and the random effects model, respectively, which supports a slightly protective effect of COX-2-inhibitors against breast cancer (Table 18, Figure 30. The fixed-effects model shows a high heterogeneity among the results from the different studies (I^2 = 91.4%), but most of this heterogeneity has been eliminated using the random-effects model (I^2 = 27.9%). Both ORs from Harris (Harris *et al.,* 2006) were far lower than the others, and were based on very few patients; therefore, to further analyze whether Harris *et al.,* 2006 would be an influential study, we performed a sensitivity analysis by deleting it; the resulting OR was virtually the same –up to the second decimal figure. Egger test (p = 0.39) and trim and fill test rejected the hypothesis of small-study or publication biases (Figures 31-33).

NSAID CONSUMPTION AND ER+ BREAST CANCER

RELATIONSHIP BETWEEN ANY NSAID AND ER+ BREAST CANCER

Three ORs from two studies (Zhang *et al.,* 2005; Kirsh *et al.,* 2007;) have been identified regarding use of any NSAID and incidence of ER+ breast tumors. The pooled OR is 0.72 (0.63-0.83) (Figure 34a), which suggests a protective effect. No study showed a relevant influence and Egger test ruled out a small-study bias (p = 0.54) (Figures 35a, 36a, 37a).

Data on NSAID use and estrogen receptor-positive (ER+) breast cancer have been found in 5 cohort studies (Marshall *et al.,* 2005; Gallichio *et al.,* 2006; Gill *et al.,* 2007; Friis *et al.,* 2008; Bardia *et al.,* 2011). The pooled RR is 0.99 (0.91-1.08) and 0.96 (0.79-1.17), using the

fixed effects model and the random effects model, respectively (Figure 34b). The heterogeneity estimated using the fixed effects model was high (I^2 = 77.1%), but most of it was eliminated using the random effects model (I^2 = 2.4%). One study appears as particularly influential (Bardia *et al.*, 2011). Egger test ruled out a small-study bias (p = 0.66) (Figures 35b, 36b, 37b).

RELATIONSHIP BETWEEN ASPIRIN AND ER+ BREAST CANCER

Data on aspirin use and risk of ER+ breast cancer were found in 3 case-control studies (Terry *et al.,* 2004; Zhang *et al.,* 2005; Kirsh *et al.,* 2007), which provided 4 ORs. The pooled OR was 0.73 (0.63-0.83) (Table 18, Figure 38a). No study showed a relevant influence and Egger test excludes the possibility of a small-study bias (p = 0.41) (Figures 39a, 40a and 41a).

Eight RRs provided by 7 cohort studies have been identified for aspirin use and estrogen-receptor-positive breast cancer (Marshall *et al.*, 2005; Gill *et al*., 2007; Friis *et al.*, 2008; Zhang *et al.,* 2008; Eliassen *et al.*, 2009; Bardia *et al.*, 2011; Zhang *et al.*, 2012), with a pooled RR of 0.94 (0.88-1.00) and 0.97 (0.87-1.07) using the fixed effects model and the random effects model, respectively (Table 18, Figure 38b). A moderate heterogeneity was detected using the fixed effects model (I^2 = 57.2%), although most of it was eliminated using the random effects model (I^2 = 3.83%) The trim and fill method suggested that two studies would have been missed; the trim and fill corrected RR (random-effect) was 0.93 (0.84-1.03). Egger test excluded the possibility of a small-study bias (p = 0.11). No study showed a relevant influence (Figures 39b, 40b, 41b).

RELATIONSHIP BETWEEN IBUPROFEN AND ER+ BREAST CANCER

Only one case control study (Zhang *et al.*, 2005) and one cohort study (Marshall *et al.,* 2005) contain data for ibuprofen use and incidence of ER+ breast cancer (OR = 0.94, 95% CI 0.44-0.91; and RR = 1.25, 95% CI 1.05-1.49, respectively). No meta-analysis has been performed.

RELATIONSHIP BETWEEN ACETAMINOPHEN AND ER+ BREAST CANCER

There are 3 cohort studies providing 4 RRs for acetaminophen use and risk of ER+ breast cancer (Marshall *et al.,* 2005; Eliassen *et al.,* 2009; Zhang *et al.,* 2012). The pooled RR is 0.93 (0.86-1.01) (Figure 42). I^2 for heterogeneity was 0.9% and Egger test excludes the

possibility of small-study effect (0.336). The trim and fill method detected that one study had been missed; when added, the corrected RR (fixed-effects) was 0.92 (0.85-1.00). No study shows a relevant influence (Figures 43-45) and Egger test excluded the possibility of a small-study bias (p = 0.32).

RELATIONSHIP BETWEEN NON-ASPIRIN NSAIDS AND ER+ BREAST CANCER

Seven RRs from 4 cohort studies (Marshall *et al.*, 2005; Friis *et al.,* 2008; Eliassen *et al.,* 2009; Bardia *et al.*, 2011; Zhang *et al.,* 2012) have been identified for non-aspirin NSAID use and risk of ER+ breast cancer, with a pooled RR of 0.99 (0.92-1.07) (Figure 46). No study shows a relevant influence and Egger test excluded the possibility of a small-study bias (p = 0.57) (Figures 47-49).

Only two ORs from one case-control study (Kirsh *et al.*, 2007) have been found: 0.85 (0.39-1.06) (ER+, PR+) and 0.72 (0.57-0.91) (ER+, PR-); and therefore no meta-analysis has been performed.

NSAID CONSUMPTION AND PR+ BREAST CANCER

RELATIONSHIP BETWEEN ANY NSAID AND PR+ BREAST CANCER

There are 2 case-control studies providing 3 ORs for use of any NSAID and risk of PR+ breast cancer (Zhang *et al.,* 2005; Kirsh *et al.,* 2007). The pooled RR is 0.73 (0.63-1.43) and no study shows a relevant influence. Egger test excluded the possibility of a small-study bias (p = 0.48).

Four cohort studies contain specific data on use of any NSAID and risk of PR+ breast cancer (Marshall *et al.*, 2005; Gallicchio *et al.*, 2007; Gill *et al.*, 2007; Friis *et al.*, 2008), with a pooled RR of 1.06 (95% CI 0.97-1.17) and 1.06 (95% CI 0.93-1.22) using the fixed effects model and the random effects model, respectively (Figure 50). No study shows a relevant influence (Figures 51-53) and Egger test ensured the absence of a small-study bias (p = 0.76).

RELATIONSHIP BETWEEN ASPIRIN AND PR+ BREAST CANCER

Data on aspirin use and risk of PR+ breast cancer were found in case-control 3 studies (Terry *et al.,* 2004; Zhang *et al.,* 2005; Kirsh *et al.,* 2007), which provided 4 ORs. The pooled OR is 0.73 (0.63-0.84) (Figure 54a). No study shows a relevant influence (Figures 55a, 56a, 57a) and Egger test excluded the possibility of a small-study bias (p = 0.28).

Five cohort studies provide data on aspirin use and PR+ breast cancer (Marshall *et al.*, 2005; Gill *et al.*, 2007; Friis *et al.*, 2008; Gierarch *et al.*, 2008; Zhang *et al.*, 2008), with a pooled RR of 0.95 (95% CI 0.89-1.03) and 0.98 (95% CI 0.86-1.10) using the fixed effects model and the random effects model, respectively (Figure 55b). The high heterogeneity found using the fixed effects model (I^2 = 56.2%) is reduced by using the random effects model (I^2 = 18.1%). No study shows a relevant influence and Egger test does not support the presence of a small-study bias (p = 0.22) (Figures 55b, 56b, 57b).

RELATIONSHIP BETWEEN IBUPROFEN AND PR+ BREAST CANCER

Only one OR (0.94, 95% CI 0.44-20.1) for ibuprofen use and PR+ cancer risk has been found (Zhang *et al*., 2005), and therefore no meta-analysis has been performed.

RELATIONSHIP BETWEEN ACETAMINOPHEN AND PR+ BREAST CANCER

Two cohort studies contain information on acetaminophen use and risk of PR+ breast cancer (Marshall *et al.*, 2005; Eliassen *et al.,* 2009), with a pooled RR of 0.99 (95% CI 0.82-1.19).

RELATIONSHIP BETWEEN NON-ASPIRIN NSAIDS AND PR+ BREAST CANCER

Two ORs (0.79, 95% CI 0.41-1.50; and 0.72, 95% CI 0.57-0.91) from the same case-control study (Kirsh *et al.*, 2007) have been found, and therefore no meta-analysis has been performed.

Five RRs for non-aspirin NSAID use and PR+ breast cancer have been identified in cohort studies (Marshall *et al.*, 2005; Gill *et al.,* 2007; Friis *et al.*, 2008; Gierarch *et al.*, 2008; Eliassen *et al.*, 2009), with a pooled RR of 1.05 (95% CI 0.96-1.14) (Figure 58). No study showd a relevant influence and Egger test ruled out the possibility of a small-study bias (p = 0.78) (Figures 59-61).

Table 15. OR of breast cancer for NSAID users versus non-users in case–control studies

Source	Country	Type of control	Type of NSAID	OR (95% CI, any intake)	OR (95% CI, highest intake)	No. of cases/no. of control subjects
Harris *et al.,* 1995	USA	Hospital	Any	1.12 (0.8-1.6)	0.58 (0.4-0.8)	744/767
Harris *et al.,* 1995	USA	Population	Any	0.65 (0.5-0.9)	0.60 (0.4-0.9)	303/906
Rosenberg, 1995	USA	Hospital	Any	0.8 (0.6-1.0)	-	4485/8391
Harris *et al.,* 1996	USA	Population	Any	0.66 (0.52-0.83)	0.60 (0.40-0.91)	511/15
Neugut *et al.,* 1998	USA	Hospital	Aspirin	0.80 (0.35-1.80)	-	252/322
Coogan *et al.,* 1999	USA	Hospital	Any	0.70 (0.60-0.90)	0.6 (0.3-1.0)	6558/2925
Langman *et al.,* 2000	UK	Hospital	Any	1.01 (0.93-1.10)	1.12 (0.90-1.40)	3105/9272
Cottercchio *et al.,* 2001	Canada	Population	Any	0.76 (0.66-0.88)	0.68 (0.54-0.86)	3133/3062
Meier *et al.,* 2002	UK	Population	Any Acetaminophen	1.00 (0.9-1.1) 1.00 (0.9-1.1)	1.0 (0.8-1.1) 0.8 (0.7-1.0)	3706/14155
Moorman *et al.,* 2003	USA	Population	Any	0.4 (0.3-0.6)	0.3 (0.2-0.5)	930/754
Terry *et al.,* 2004	USA	Population	Aspirin Ibuprofen Acetaminophen	0.80 (0.66-0.97) 0.91 (0.72-1.16) 1.02 (0.80-1.31)	0.77 (0.57-1.04) 1.09 (0.70-1.70) 0.91 (0.58-1.41)	1442/1420 1443/1420 1434/1417
Rahme *et al.,* 2005	Canada	Population	Cox-2- inhibitors Non-aspirin NSAIDs Aspirin Acetaminophen	0.81 (0.68-0.97) 0.65 (0.43-0.99) 0.75 (0.64-0.89) 0.91 (0.71-1.16)	- - - -	1090/44990
Swede *et al.,* 2005	USA	Hospital	Aspirin	0.83 (0.75-0.93)	0.85 (0.75-0.96)	1478/3383
Zhang *et al.,* 2005	USA	Hospital	Any	1.01 (0.90-1.13)	0.62 (0.28-1.35)	7006/3622

Harris *et al.,* 2006	USA	Hospital	Cox-2-inhibitors Aspirin Baby aspirin Ibuprofen/naproxen Acetaminophen	0.29 (0.14-0.59) 0.49 (0.26-0.94) 0.82 (0.40-1.40) 0.37 (0.18-0.72) 1.02 (0.39-2.20)	- 0.39 (0.22-0.72) - - -	323/649
Davis y Mirick, 2007	USA	Population	Any	1.1 (0.8-1.4)	1.0 (0.7-1.5)	600/647
Kirsh *et al.,* 2007	Canada	Population	Any	0.76 (0.66-0.88)	-	3125/3062
Slattery *et al.,* 2007	USA	Population	Aspirin	0.94 (0.82-1.07)	-	2325/2525
Brasky *et al.,* 2010	USA	Population	Aspirin Ibuprofen Acetaminophen	0.80 (0.68-0.94) 1.15 (0.97-1.36) 0.97 (0.83-1.15)	0.68 (0.46-1.00) 1.12 (0.94-1.34) 1.01 (0.85-1.20)	1170/2115
Cronin-Fenton *et al.,* 2010	Denmark	Population	Any	1.04 (0.99-1.10	1.01 (0.52-1.97)	8195/81950
Ashok *et al.,* 2011	USA	Population	Non-selective NSAIDs Celecoxib Rofecoxib Valdecoxib Acetaminophen	0.85 (0.82-0.88) 0.86 (0.81-0.91) 0.68 (0.62-0.74) 0.81 (0.71-0.9) 0.95 (0.85-1.06)	0.78 (0.69-0.89) 0.84 (0.73-0.97) 0.59 (0.46-0.76) 0.94 (0.52-1.68) 1.09 (0.61-1.92)	18368/73472
Vinogradova *et al.,* 2011	UK	Population (nested)	Cox-2-inhibitors	1.24 (1.08-1.42)	1.19 (0.98-1.44)	15666/88125
Ou *et al.,* 2013	Taiwan	Hospital (nested)	Any	0.41 (0.19-0.89)	-	11/36

Table 16. RR of breast cancer for NSAID users versus non-users in cohort studies or randomized controlled trials

Source	Country	Type of NSAID	RR (95% CI, any intake)	RR (95% CI, highest intake)	No. of cases/cohort size
Paganini-Hill *et al.,* 1989	USA	Aspirin	0.96	-	214/8818
Schreinemachers & Everson, 1994	USA	Aspirin	0.72 (0.52-1.00)	-	174/11411
Egan *et al.,* 1996	USA	Aspirin	1.01 (0.80-1.27)	1.12 (0.76-1.66)	2414/89528
Harris *et al.,* 1999	USA	Any Aspirin Acetaminophen Ibuprofen	0.64 (0.50-0.82) 0.57 (0.40-0.81), 0.84 (0.55-1.18) 0.53 (0.33-0.84)	0.57 (0.44-0.74) 0.64 (0.45-0.90) 0.84 (0.47-1.50) 0.49 (0.30-0.80)	393/32505 76/32505 36/32505 37/32505
Sharpe *et al.,* 2000	USA	Any	0.95 (0.91-0.99)	0.91 (0.75-1.09)	5882/25317
Friis *et al.,* 2002	Denmark	Acetaminophen	1.0 (0.9-1.2)	-	227/39946
Johnson *et al.,* 2002	USA	Any	0.80 (0.67-0.95)	1.01 (0.83-1.25)	938/27616
Friis *et al.,* 2003	Denmark	Aspirin	0.9 (0.8-1.1)	-	149/29470
Harris *et al.,* 2003	USA	Any	0.93 (0.78-1.10)	0.81 (0.68-0.97)	1392/80741
Sorensen *et al.,* 2003	Denmark	Any	1.1 (1.0-1.2)	1.1 (0.9-1.3)	696/172057
Ratnasinghe *et al.,* 2004	USA	Aspirin	0.82 (0.49-1.36)	-	131/12834
García-Rodríguez y González-Pérez, 2004	UK, Spain	Aspirin Non-aspirin NSAIDs Acetaminophen	0.84 (0.69-1.02) 0.98 (0.88-1.09) 0.92 (0.83-1.03)	0.87 (0.53-1.41) 1.05 (0.80-1.38) 0.76 (0.60-0.97)	3708/734899
Cook *et al.,* 2005; Zhang *et al.,* 2008	UK	Aspirin	0.98 (0.87-1.09)	-	1230/39884
Jacobs *et al.,* 2005	USA	Any	1.16 (1.02-1.31)	1.05 (0.88-1.26)	3008/77413
Marshall *et al.,* 2005	USA	Any Acetaminophen Ibuprofen Aspirin	- - - -	1.11 (0.96-1.30) 0.96 (0.63-1.47) 1.51 (1.17-1.95) 0.96 (0.79-1.18)	2391/114640

Gallichio *et al.,* 2007	USA	Any Acetaminophen	0.89 (0.72-1.09) 0.94 (0.71-1.25)	- -	418/15651
Gill *et al.,* 2007	USA	Any Acetaminophen	0.88 (0.75-1.04) 1.14 (0.91-1.42)	0.99 (0.82-1.18) 1.05 (0.83-1.33)	3493/98920 278/98920
Jacobs *et al.,* 2007	USA	Aspirin	1.02 (0.88-1.19)	0.83 (0.63-1.10)	3121/76303
Bardia *et al.,* 2007	USA	Aspirina Non-aspirin NSAIDs Combined use	0.84 (0.77-0.90) 0.96 (0.89-1.04) 0.81 (0.72-0.90)	0.81 (0.73-0.90) 0.94 (0.83-1.06) -	3487/22507
Friis *et al.,* 2008	Denmark	Any	1.34 (1.17–1.54)		847/28695
Ready *et al.,* 2008	USA	Any	0.99 (0.82-1.19)	0.91 (0.75-1.09)	482/35323
Gierarch *et al.,* 2008	USA	Any	0.95 (0.87-1.04)	-	4501/126124
Siemes *et al.,* 2008	Netherlands	Any	1.19 (0.81-1.73)	1.27 (0.80-2.00)	175/7621
Eliassen *et al.,* 2009	USA	Aspirin Non-aspirin NSAIDs Acetaminophen	1.07 (0.89-1.29) 1.16 (1.01-1.34) 0.99 (0.84-1.16)	1.03 (0.74-1.42) 0.86 (0.60-1.24) 1.06 (0.64-1.76)	1345/112292
Bardia *et al., 2*011	USA	Aspirin Non-aspirin NSAIDs Combined use	0.80 (0.71-0.90) 0.95 (0.85-1.07) 0.77 (0.65-0.91)	0.71 (0.60-0.83) 1.00 (0.84-1.19) -	1581/26580
Zhang *et al.,* 2012	USA	Aspirin Non-aspirin NSAIDs Acetaminophen	0.91 (0.81-1.01) 0.97 (0.90-1.04) 0.89 (0.83-0.96)		4734/84602

Table 17. RR of breast cancer for NSAID users vs. non-users in previous meta-analysis

Source	Type of NSAID	RR (95% CI)
Khuder *et al.,* 2001	Any Cohort studies Case-control studies	 0.78 (0.62-0.99) 0.87 (0.84-0.91)
González-Pérez *et al.,* 2003	Any Aspirin Non-aspirin NSAIDs	0.77 (0.66-0.88) 0.77 (0.69-0.86) 0.86 (0.73-1.00)
Bosetti *et al.,* 2006	Aspirin	0.91 (0.88-0.95)
Mangiapane *et al.,* 2008	Aspirin	0.75 (0.64-0.88)
Takkouche *et al.,* 2008	Any	0.88 (0.84-0.93)
Harris *et al.,* 2009	OTC NSAIDs	0.75 (0.67-0.84)
Zhao *et al.,* 2009	Any Aspirin Ibuprofen	0.94 (0.88–1.00) 0.91 (0.83–0.98) 0.81 (0.67–0.97)
Bosetti *et al.,* 2011	Aspirin	0.90 (0.85-0.95)
Luo *et al.,* 2012	Aspirin	0.86 (0.81-0.92)
Tolentino *et al.,* 2012	Non-aspirin NSAIDs	-
Algra *et al.,* 2013	Aspirin Case-control studies RCTs Cohort studies	 0.88 (0.82-0.95) 1.17 (0.50-2.71) -

Table 18. Results from this meta-analysis

NSAID	Receptor	Type of study	OR/RR	95%CI	I^2(%)
Any NSAID	Any	Cohort	0.92	0.84 – 1.01	89.9
	Any	Case-control	0.82	0.77 – 0.88	86.1
	Estrogen +	Cohort	0.96	0.79 – 1.17	77.1
	Estrogen +	Case-control	0.72[a]	0.63 – 0.83[a]	0
Aspirin	Any	Cohort	1.00	0.96 – 1.04	11.7
	Any	Case-control	0.87	0.82 – 0.92	4.5
	Estrogen +	Cohort	0.94	0.88 – 1.00	57.2
	Estrogen +	Case-control	0.73	0.63 – 0.83	0
	Progesterone +	Case-control	0.73	0.63 – 0.84	0
Ibuprofen	Any	Cohort	1.09[b]	0.99 – 1.20[b]	-
	Any	Case-control	0.83	0.69 – 1.00	72.5
	Estrogen +	Cohort	1.25[b]	1.05 – 1.49[b]	-
COX-2 inhibitors	Any	Case-control	0.90	0.87 – 0.93	91.4
Acetaminophen	Any	Cohort	0.95[a]	0.88 – 1.01[a]	0.75
	Any	Case-control	0.85	0.76 – 0.95	63.2
	Estrogen +	Cohort	0.92	0.85 – 1.00	0.9
Non-aspirin NSAID	Any	Cohort	1.03	0.99 – 1.08	43.6
	Any	Case-control	1.02[a]	0.98 – 1.07[a]	3.1
	Estrogen +	Cohort	0.99	0.92 – 1.07	16.2

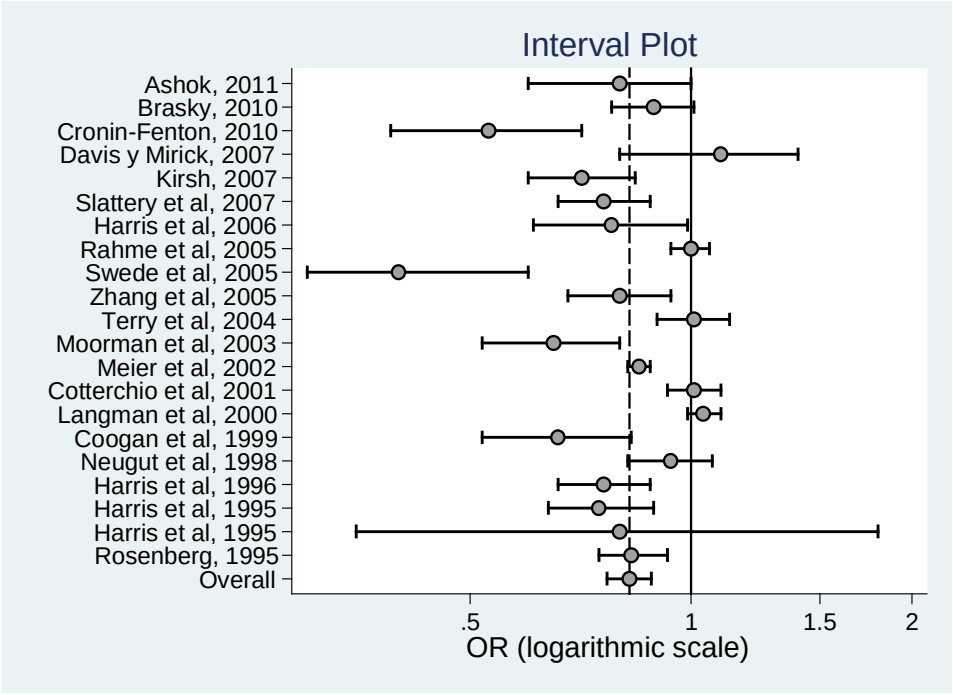
[a] *Based on two studies*

[b] *Based on one study*

I^2 *percent of the effect variability due to between studies heterogeneity*

Figure 11. Forest plot for the relationship between any NSAID and breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

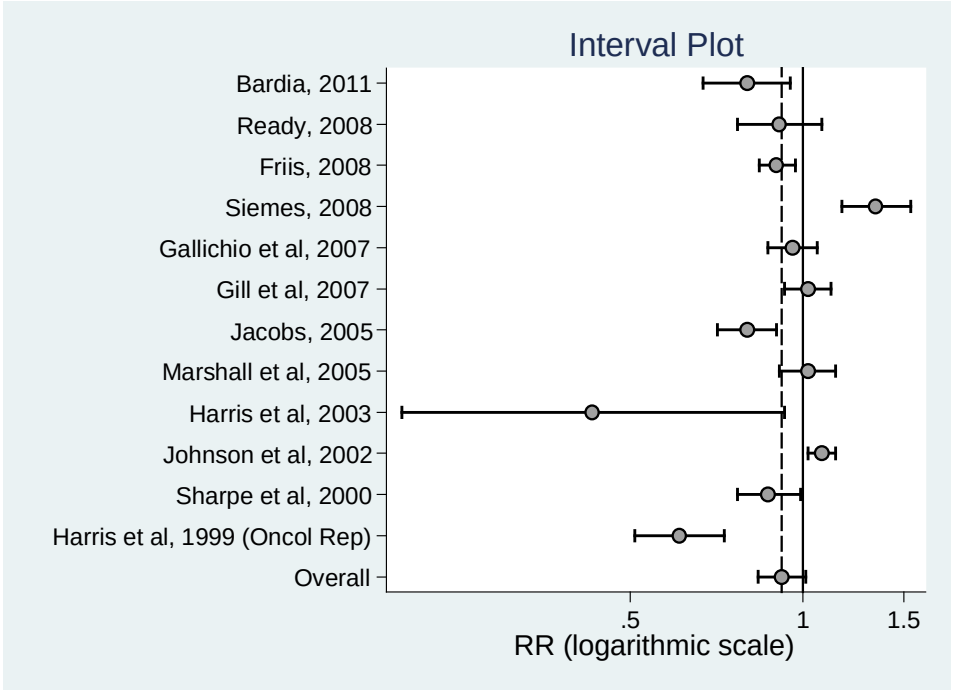
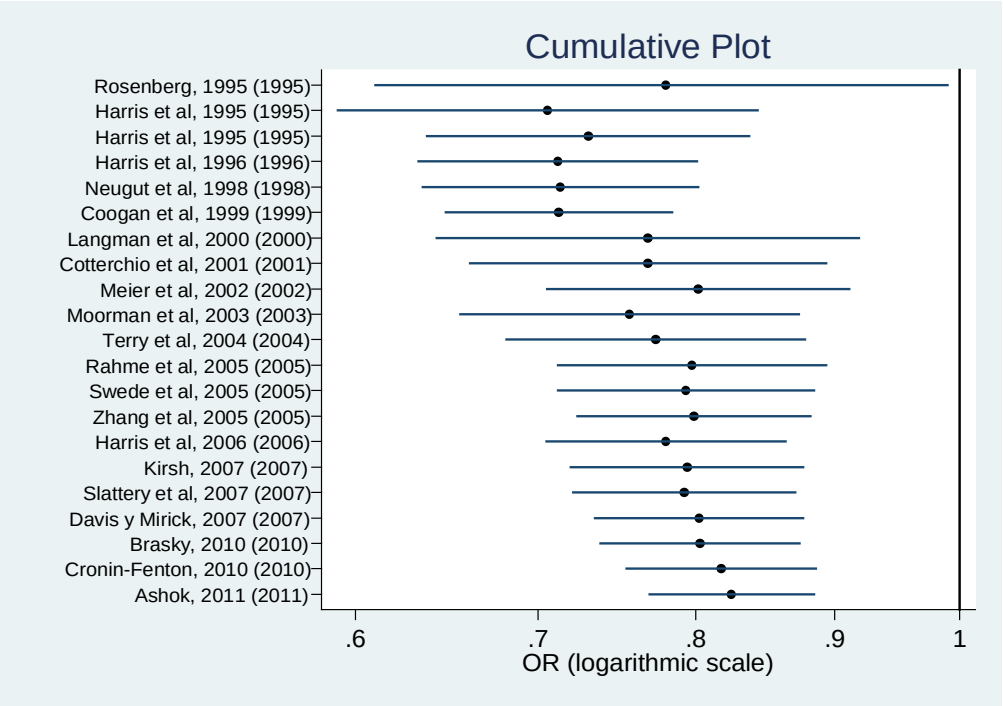


Figure 12. Cumulative meta-analysis forest plot for the relationship between any NSAID and breast cancer. a) Case-control studies; b) cohort studies

a)



b)

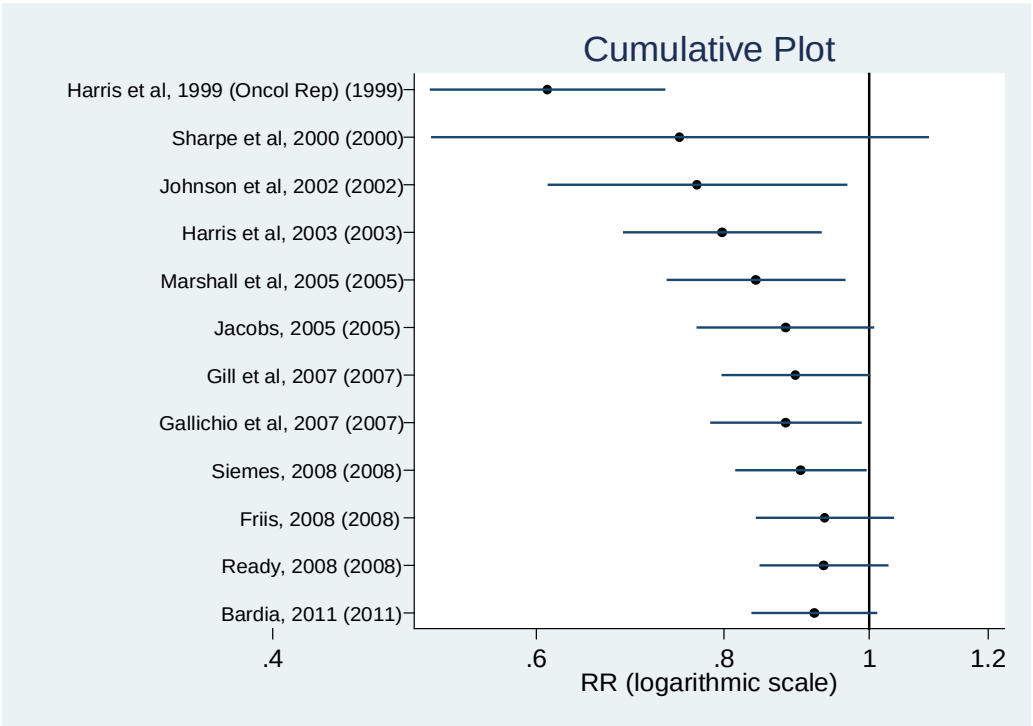
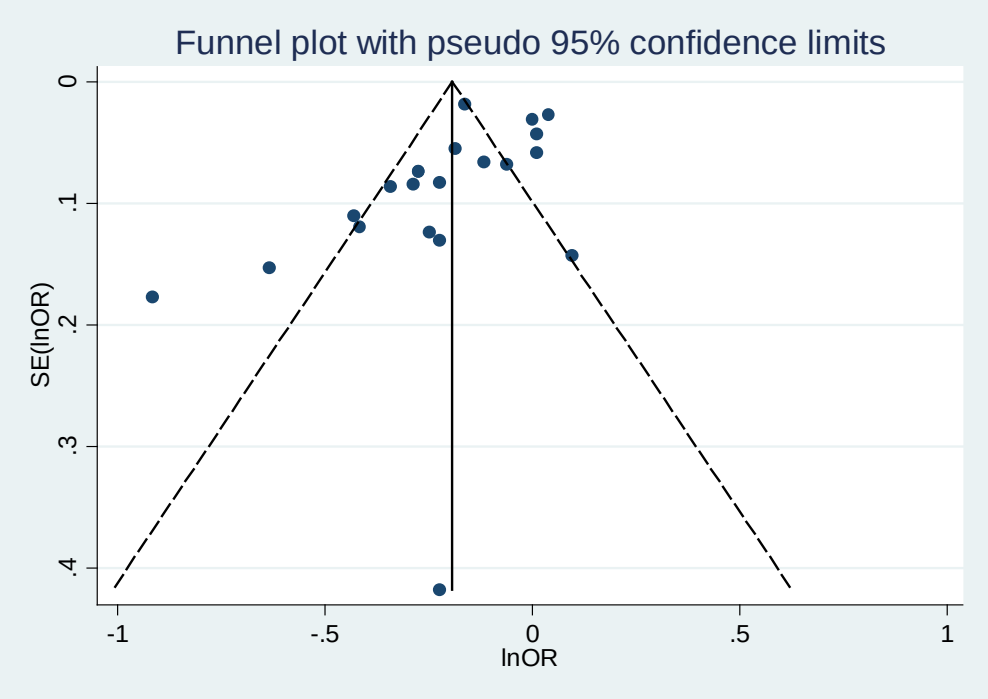


Figure 13. Funnel plot for the relationship between any NSAID and breast cancer. a) Case-control studies; b) cohort studies

a)



b)

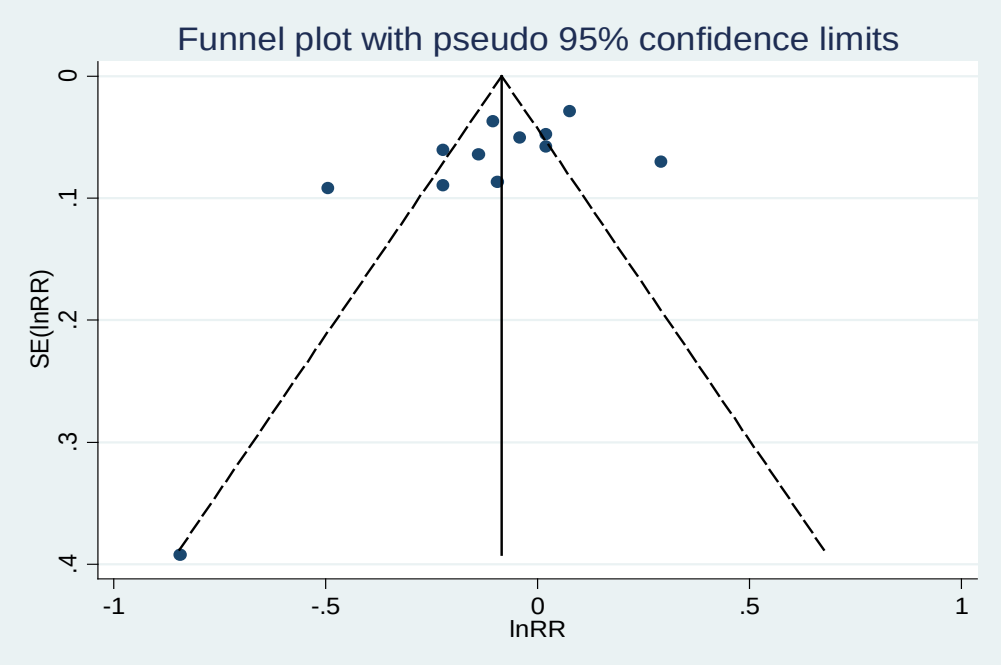
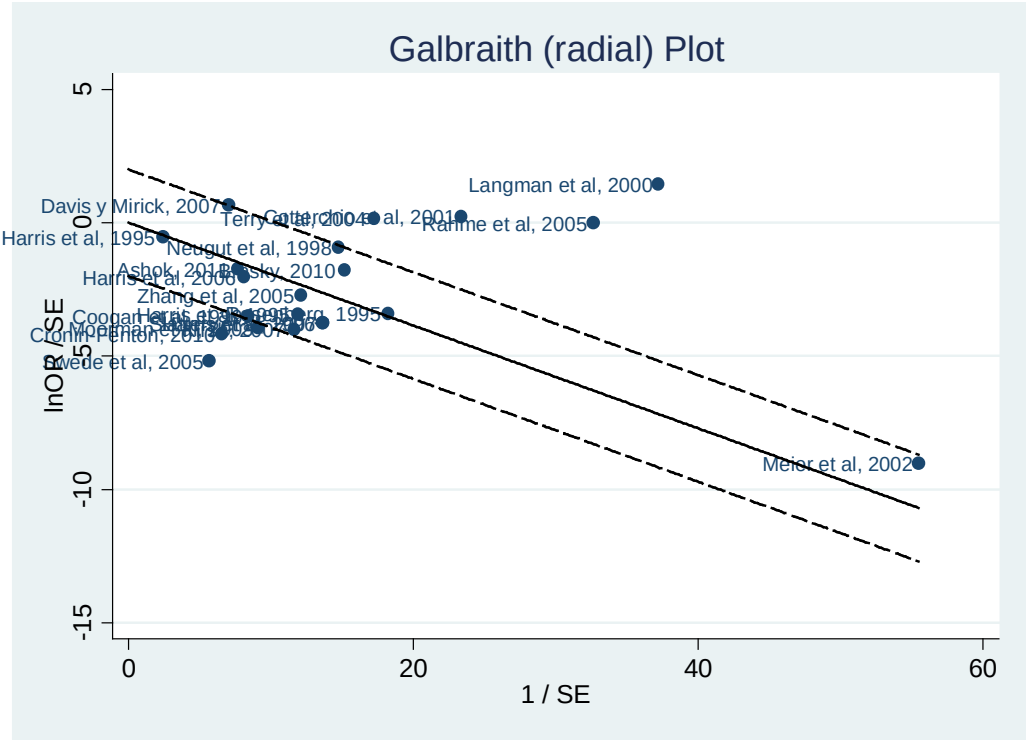


Figure 14. Galbraith radial plot for the relationship between NSAID use and breast cancer risk, with confidence bands. a) Case-control studies; b) cohort studies.

a)



b)

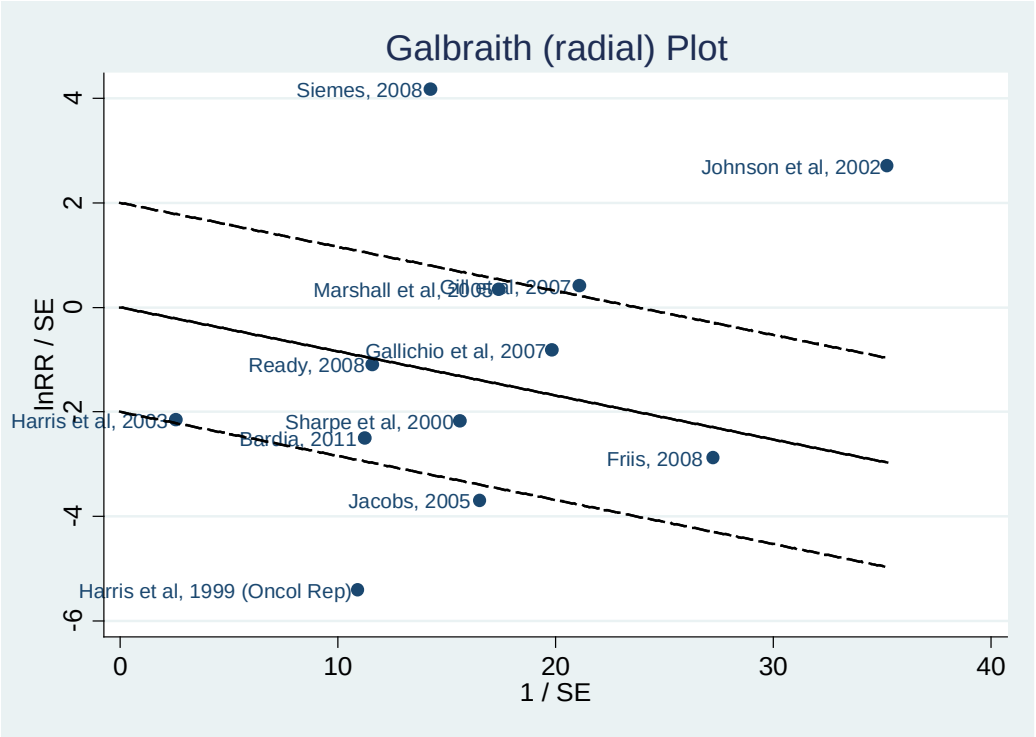
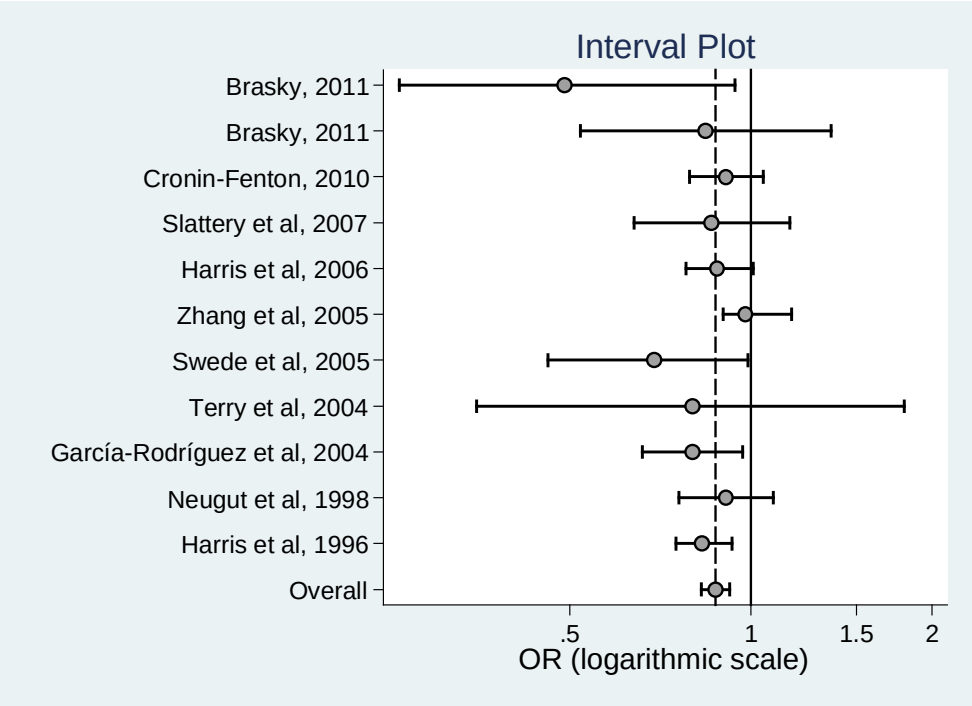


Figure 15. Forest plot for the relationship between aspirin and breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

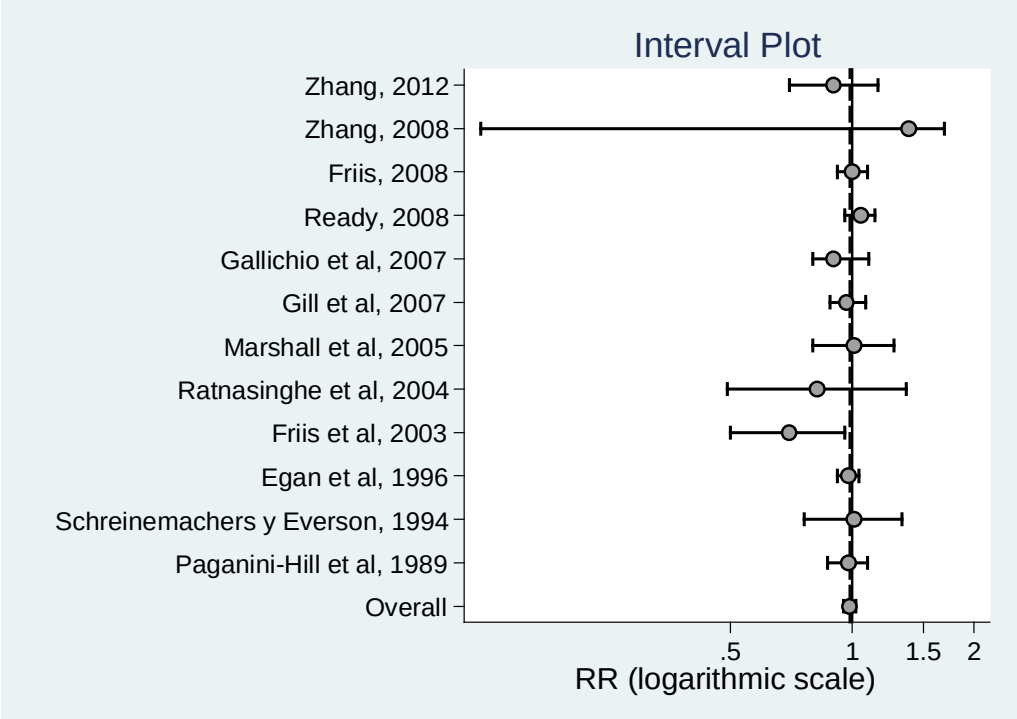
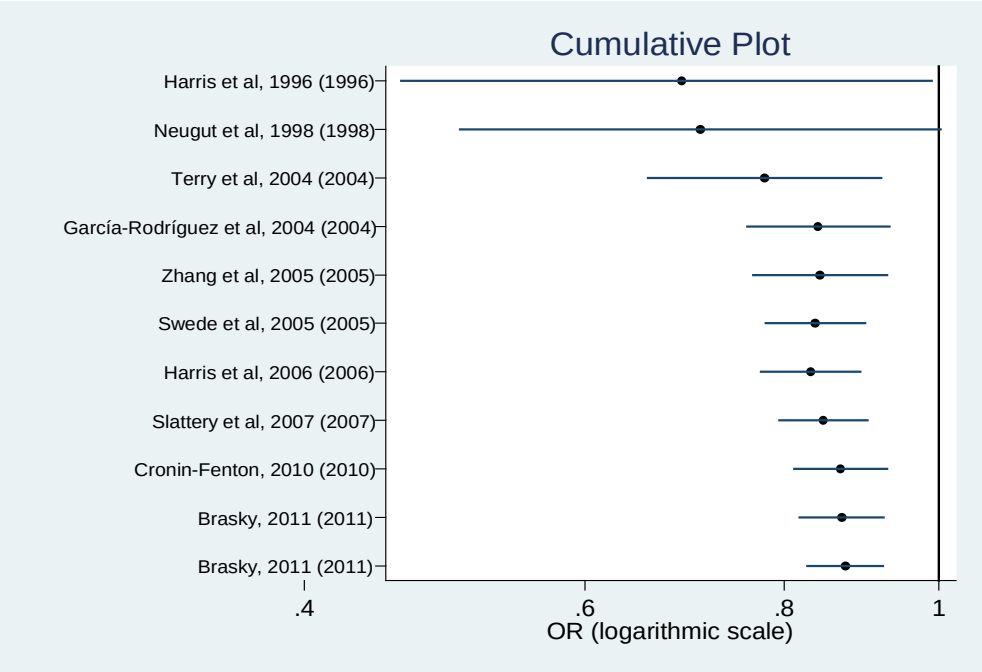


Figure 16. Cumulative meta-analysis forest plot for the relationship between aspirin and breast cancer risk. a) Case-control studies; b) Cohort studies.

a)



b)

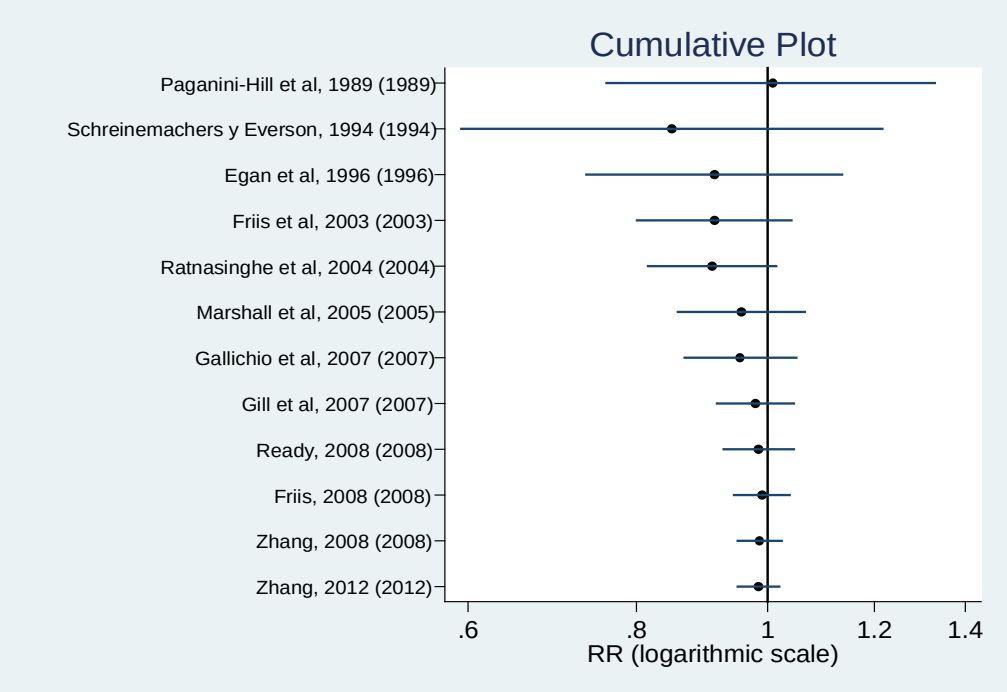
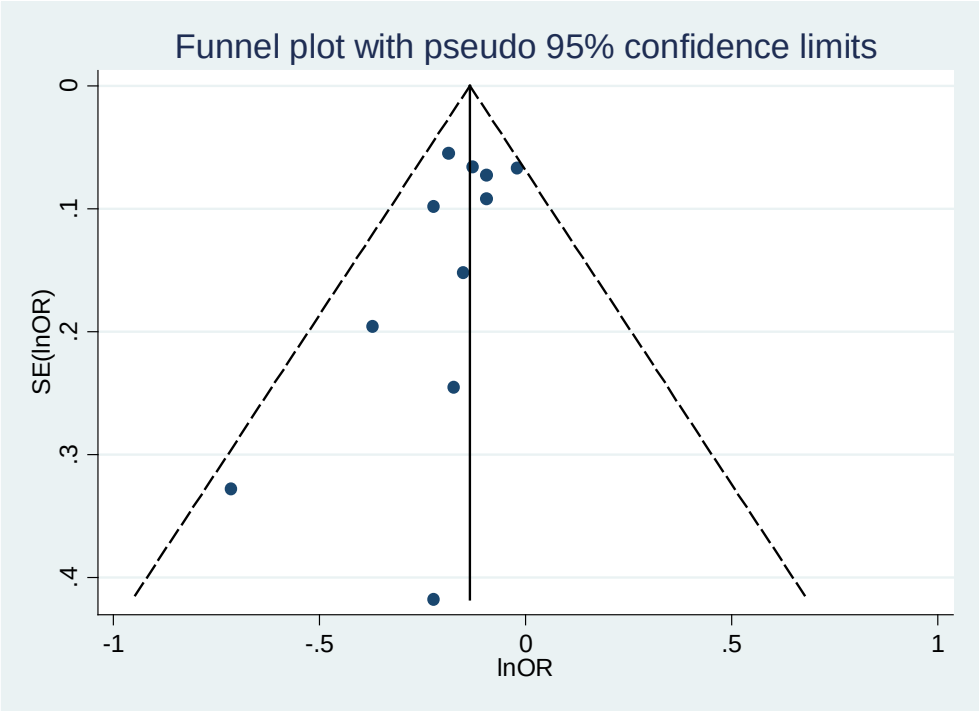


Figure 17. Funnel plot for the relationship between aspirin and breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

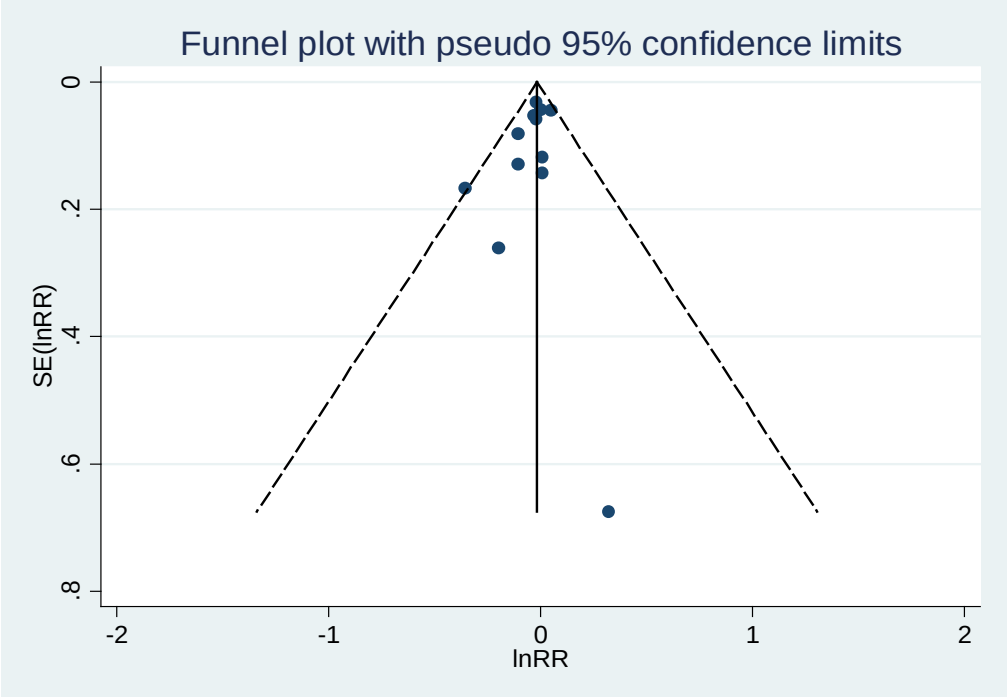
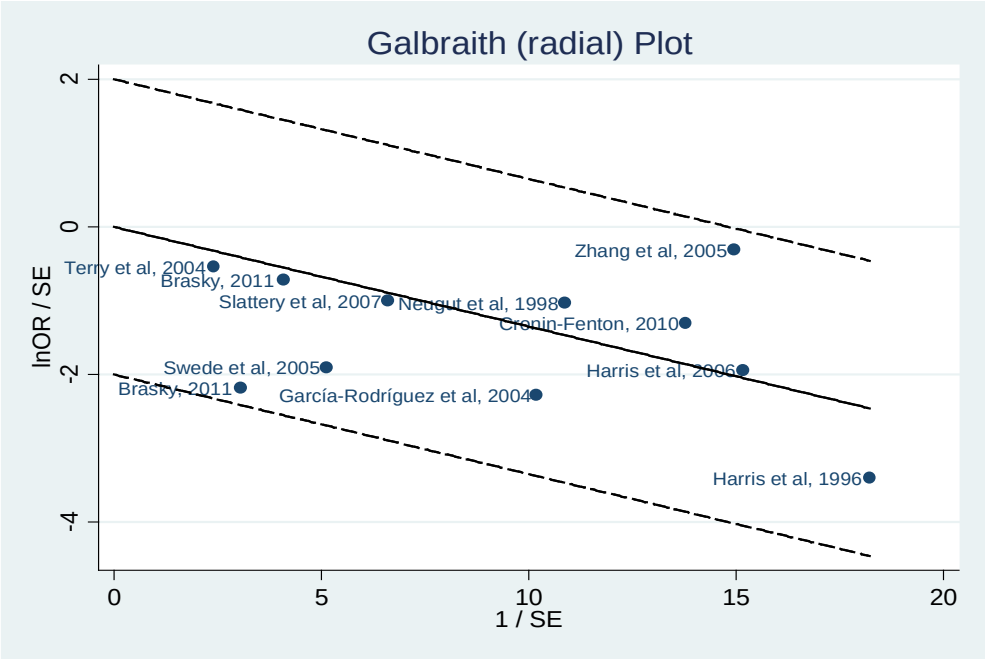


Figure 18. Galbraith radial plot for the relationship between aspirin and breast cancer risk. with confidence bands. a) Case-control studies; b) Cohort studies.

a)



b)

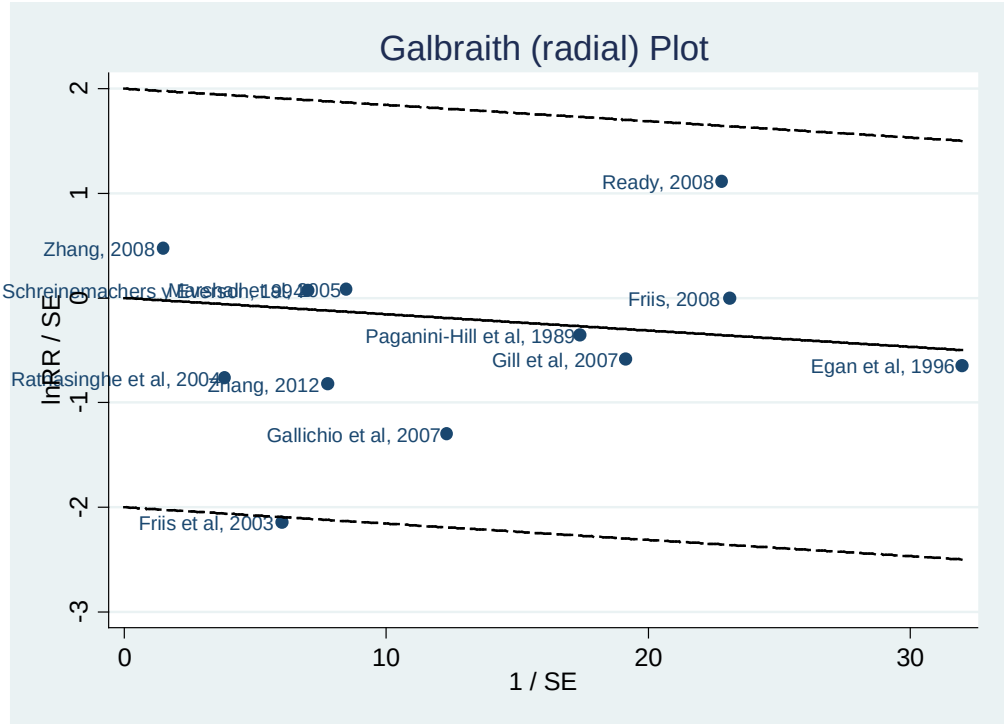


Figure 19. Forest plot for the relationship between ibuprofen and breast cancer (case control studies only).

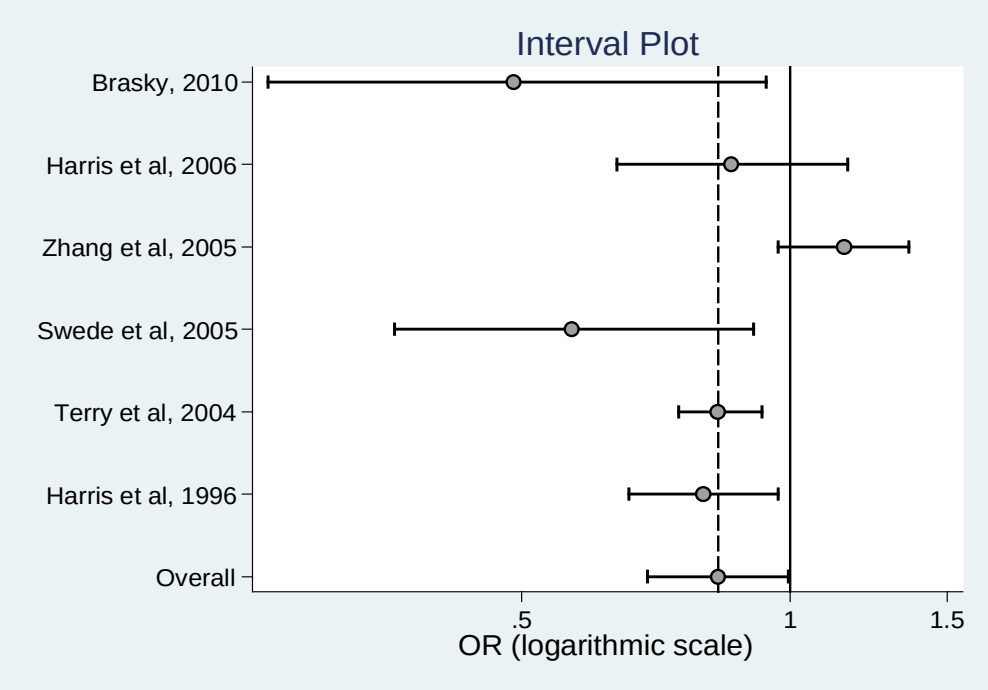


Figure 19. Cumulative meta-analysis forest plot for the relationship between ibuprofen and breast cancer (case-control studies only)

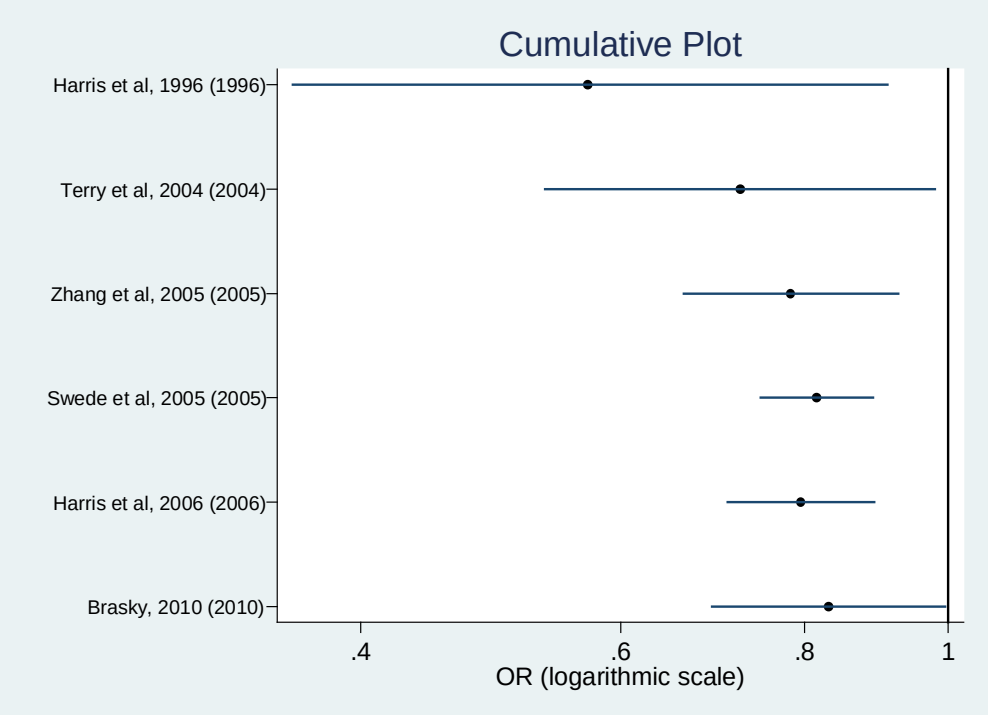


Figure 20. Funnel plot for the relationship between ibuprofen and breast cancer (case-control studies only)

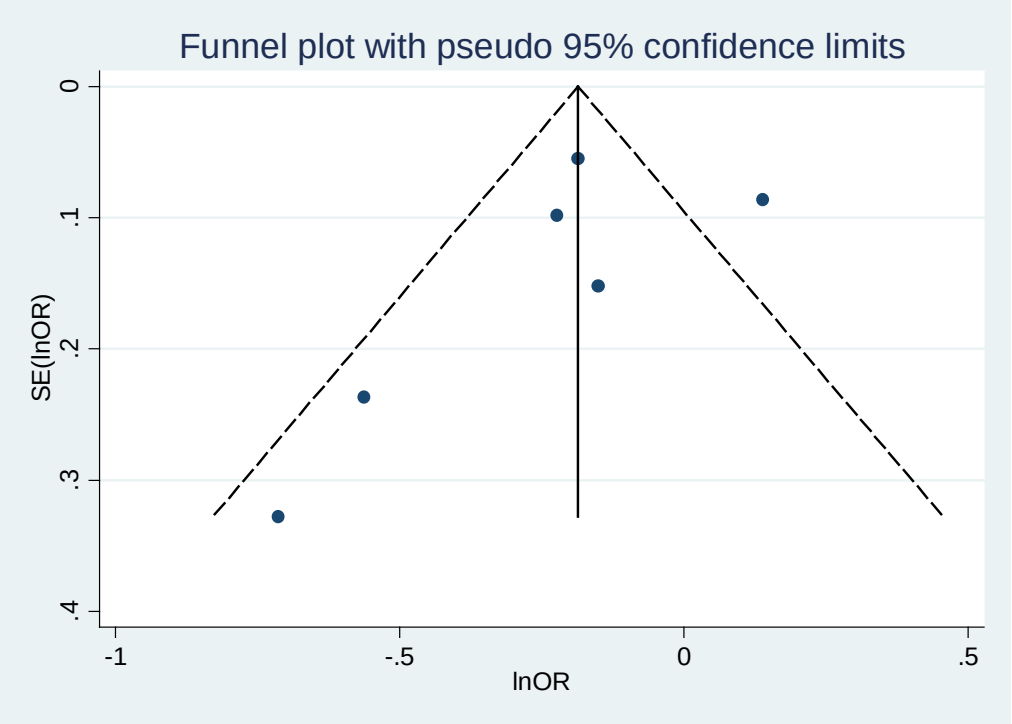


Figure 21. Galbraith radial plot for the relationship between ibuprofen and breast cancer, with confidence bands (case-control studies only)

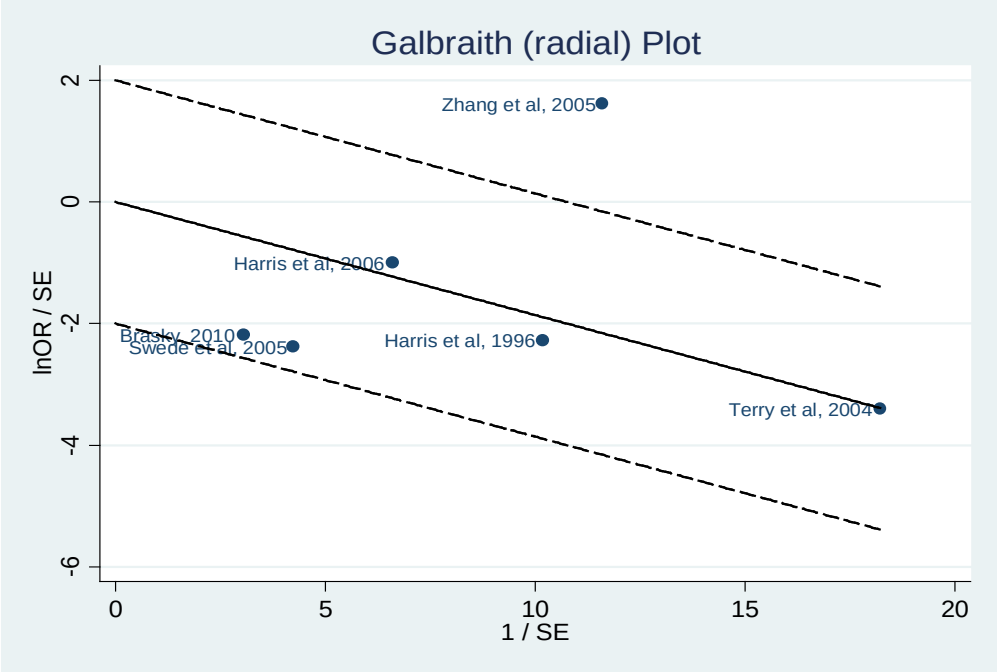


Figure 22. Forest plot for the relationship between acetaminophen and breast cancer risk (case-control studies only)

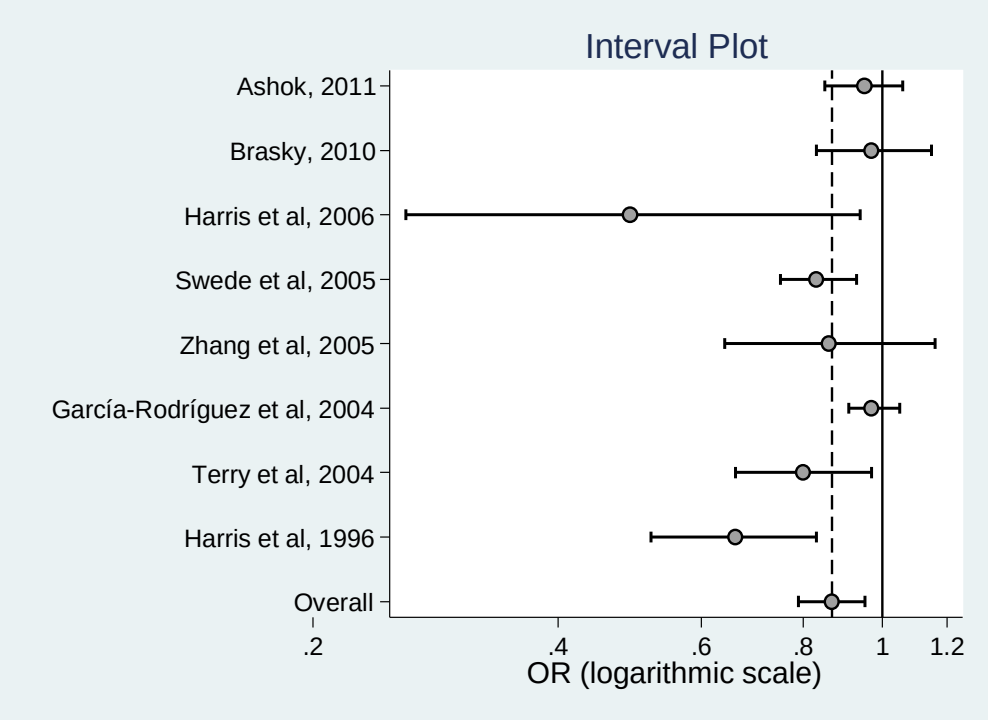


Figure 23. Cumulative meta-analysis forst plot for the relationship between acetaminophen and breast cancer (case-control studies only)

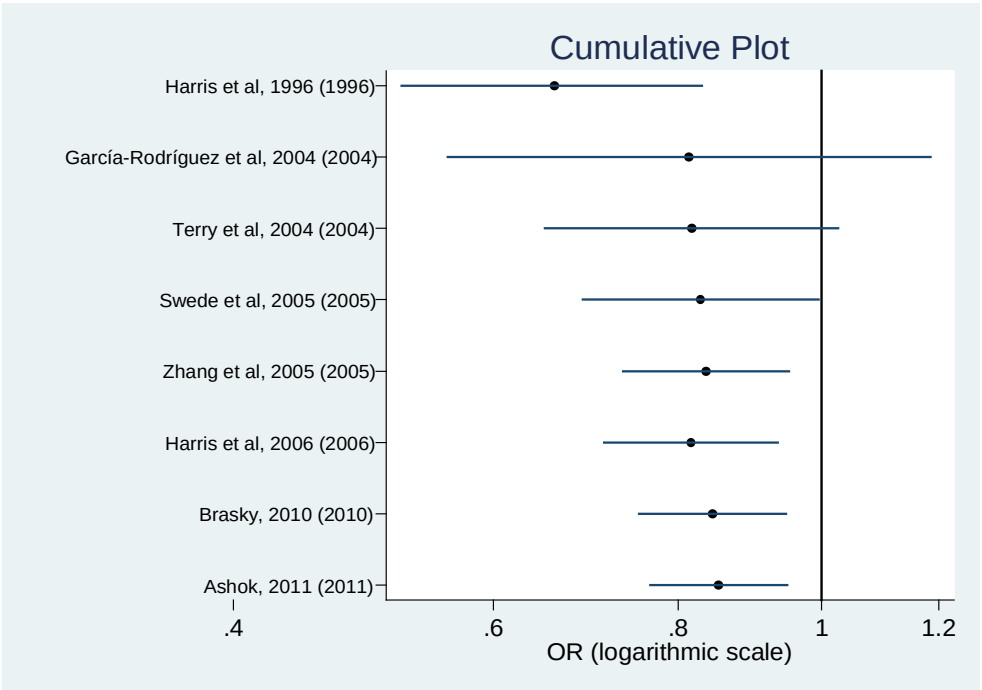


Figure 24. Funnel plot for the relationship between acetaminophen and breast cancer (case-control studies only)

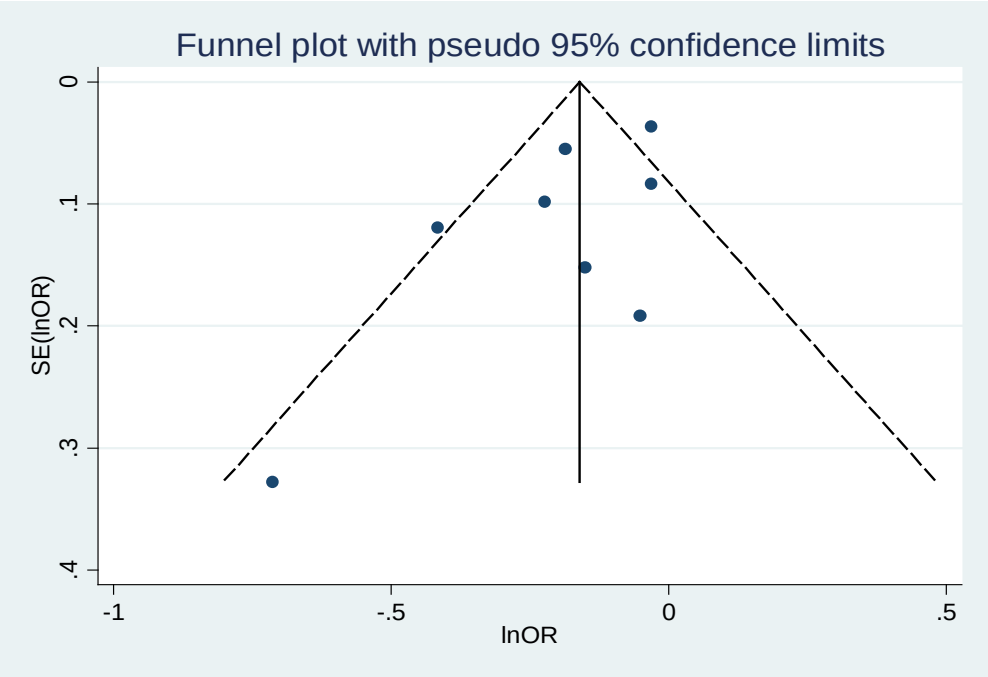


Figure 25. Galbraith radial plot for the relationship between acetaminophen and breast cancer risk (case-control studies only)

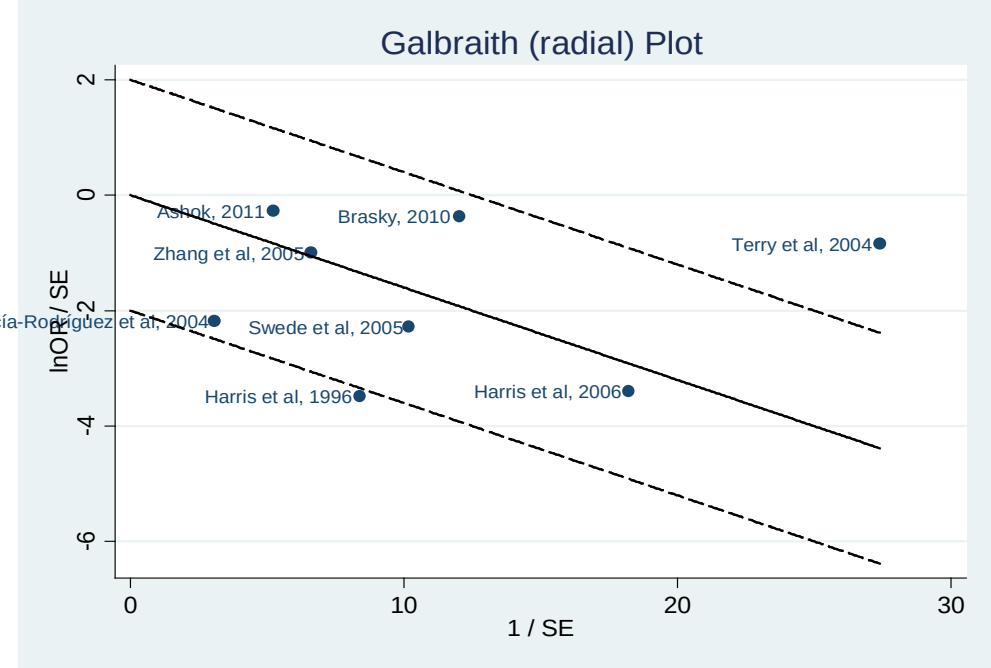


Figure 26. Forest plot for the relationship between non-aspirin NSAIDs and breast cancer (cohort studies only).

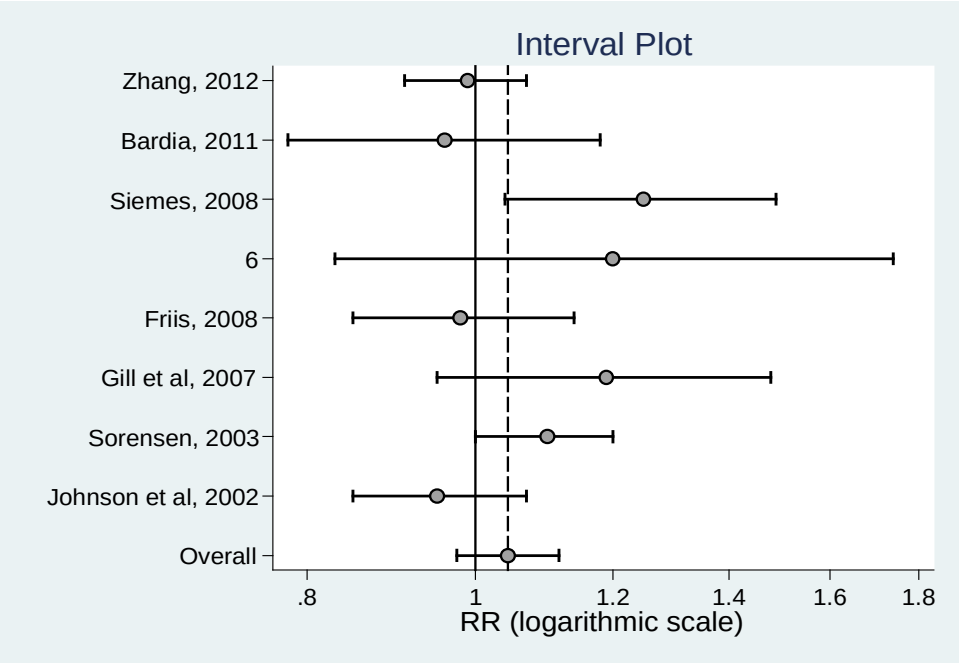


Figure 27. Cumulative meta-analysis forest plot for the relationship between non-aspirin NSAIDs and breast cancer (cohort studies only)

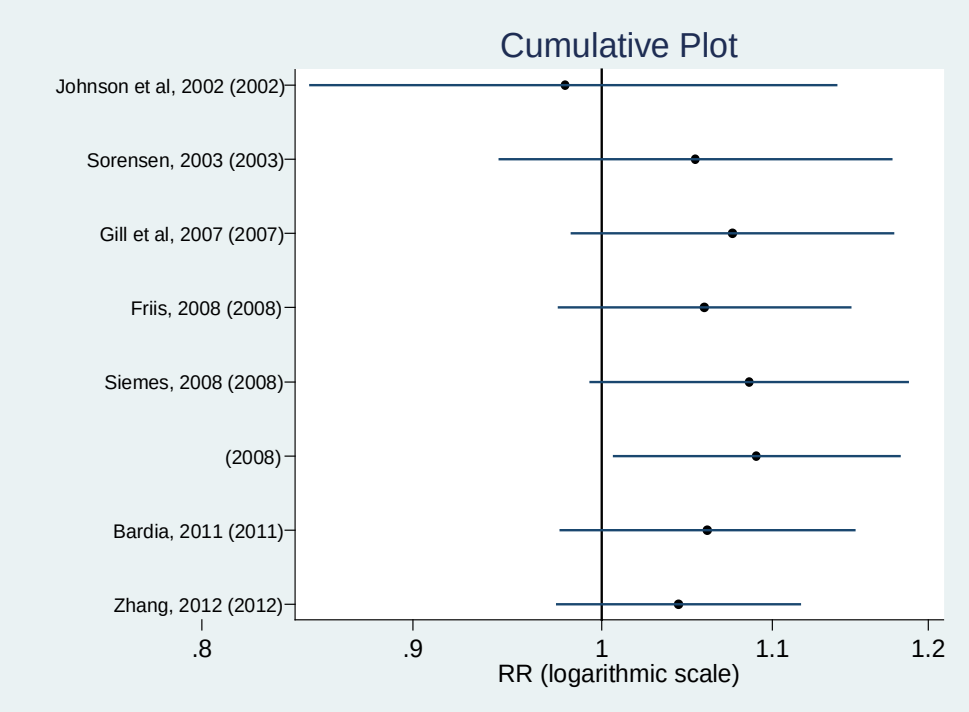


Figure 28. Funnel plot for the relationship between non-aspirin NSAIDs and breast cancer (cohort studies only)

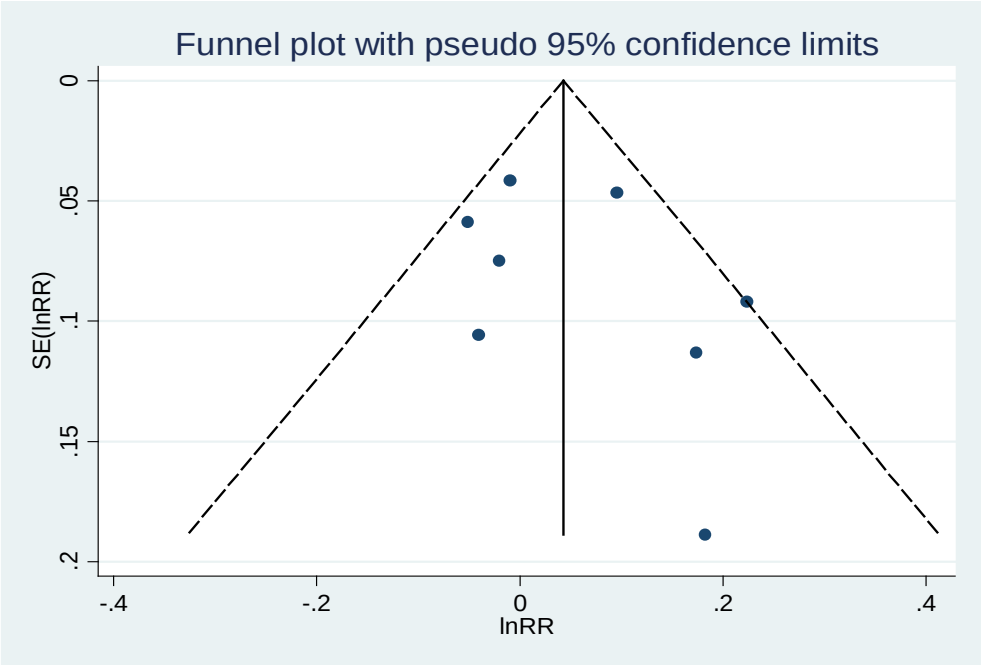


Figure 29. Galbraith radial plot for the relationship between non-aspirin NSAIDs and breast cancer risk (cohort studies only)

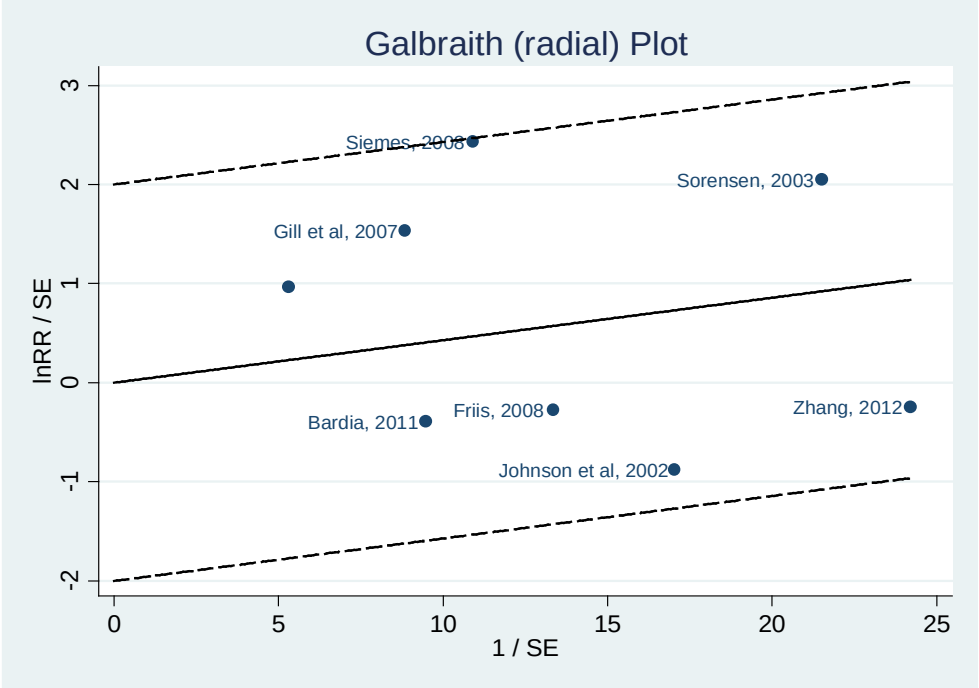


Figure 30. Forest plot for the relationship between Cox-2 inhibitors and breast cancer risk (case-control studies only)

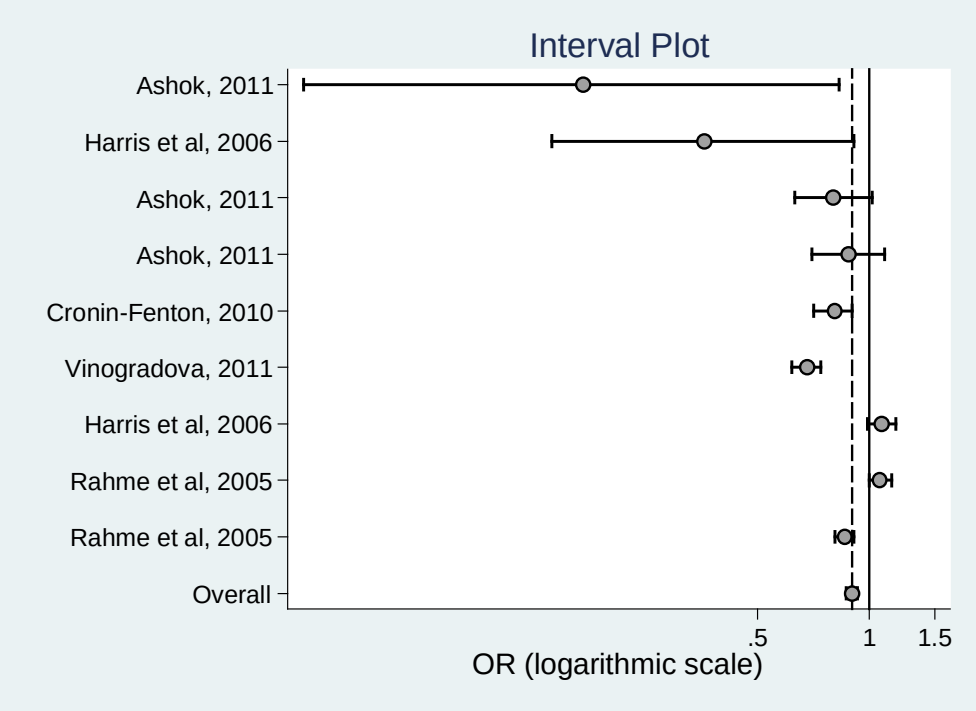


Figure 31. Cumulative plot for the relationship between Cox-2 inhibitors and breast cancer (case-control studies only).

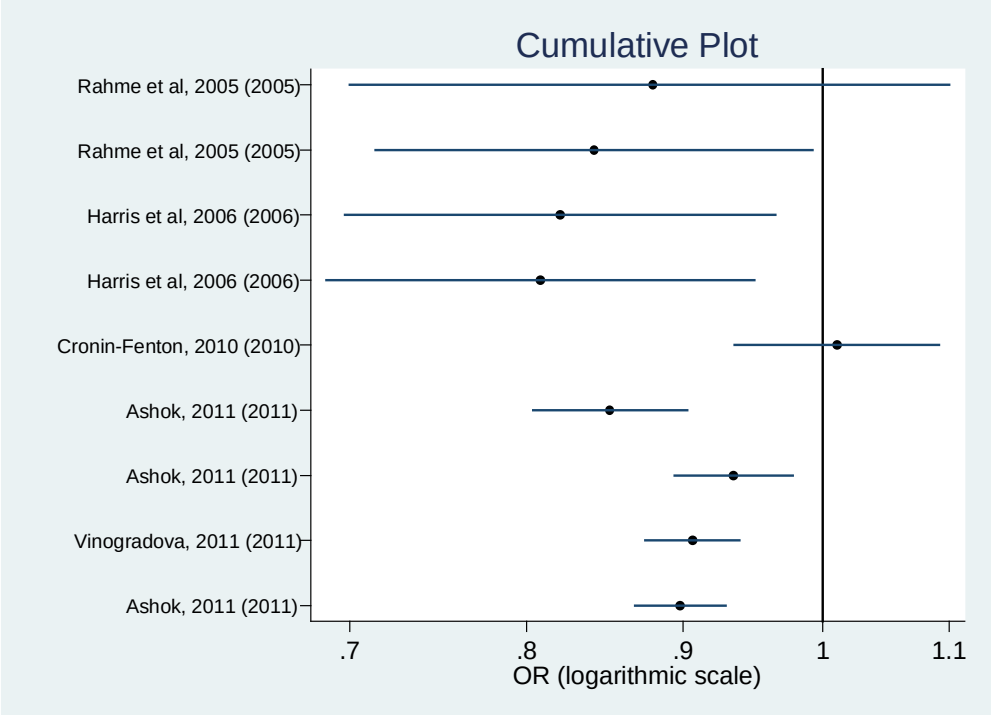


Figure 32. Funnel plot for the relationship between Cox-2 inhibitors and breast cancer (case-control studies only)

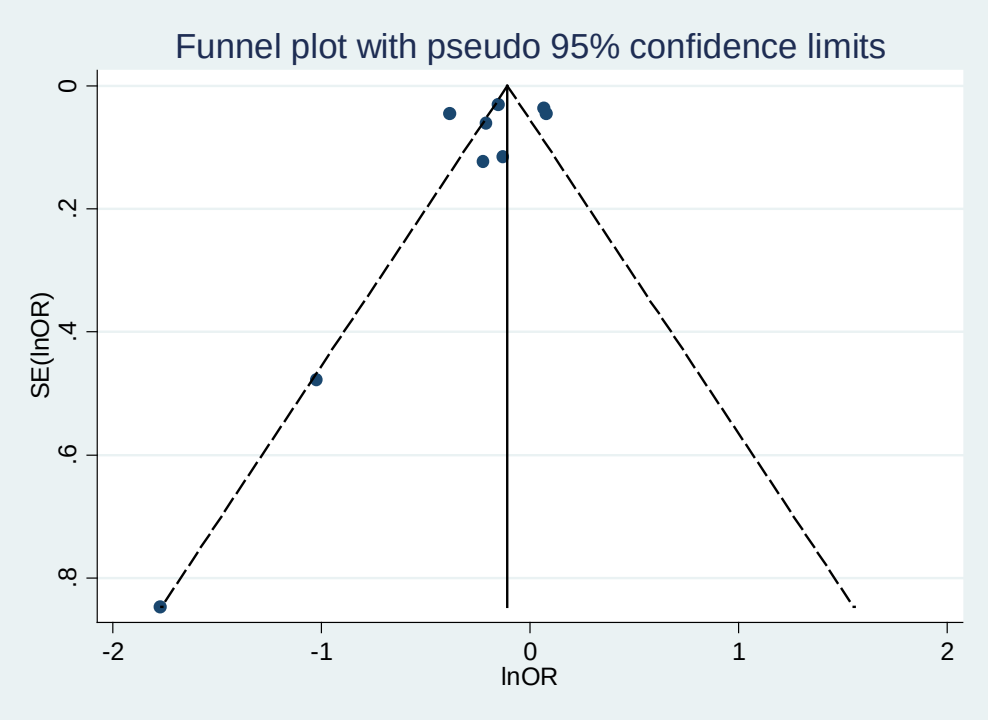


Figure 33. Galbraith radial plot for the relationship between Cox-2 inhibitors and breast cancer (case-control studies only).

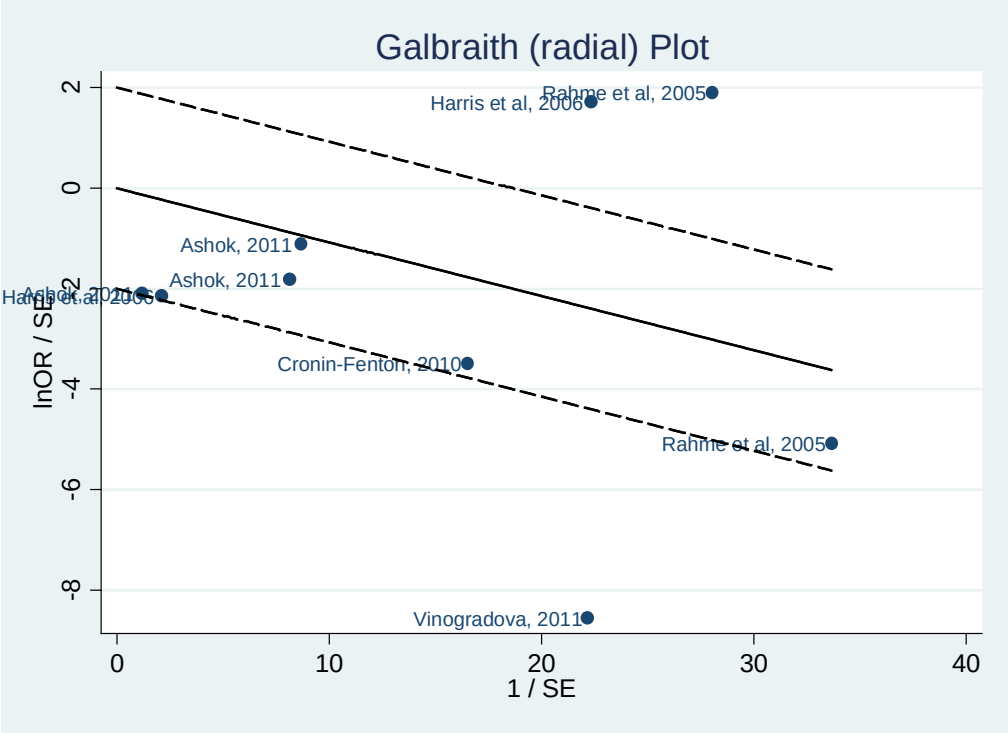
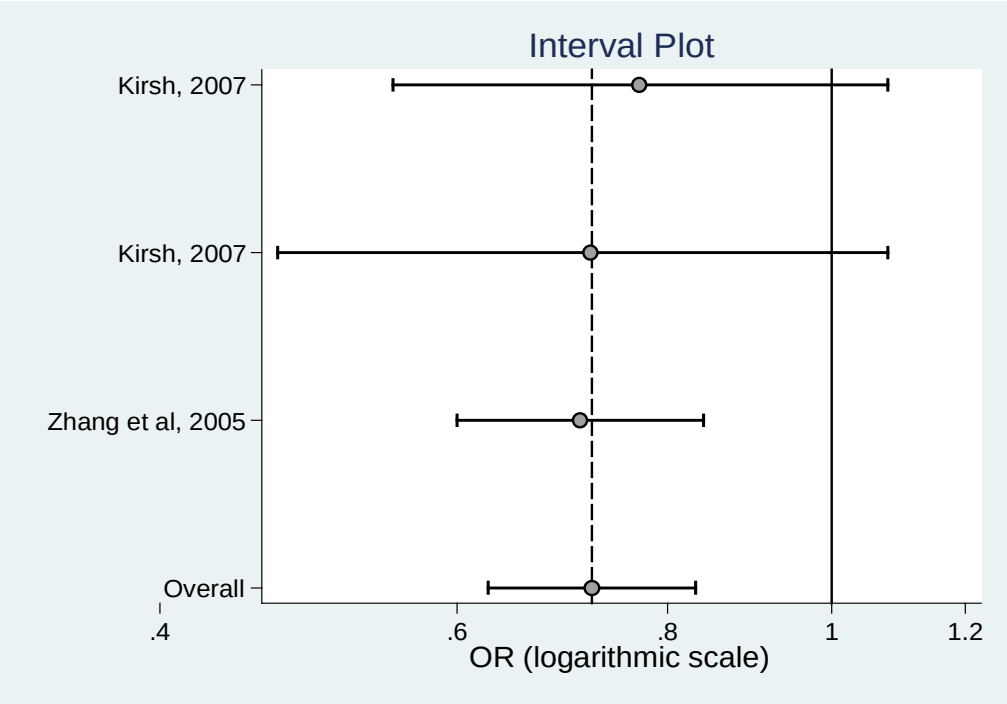


Figure 34. Forest plot for the relationship between any NSAID and ER+ breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

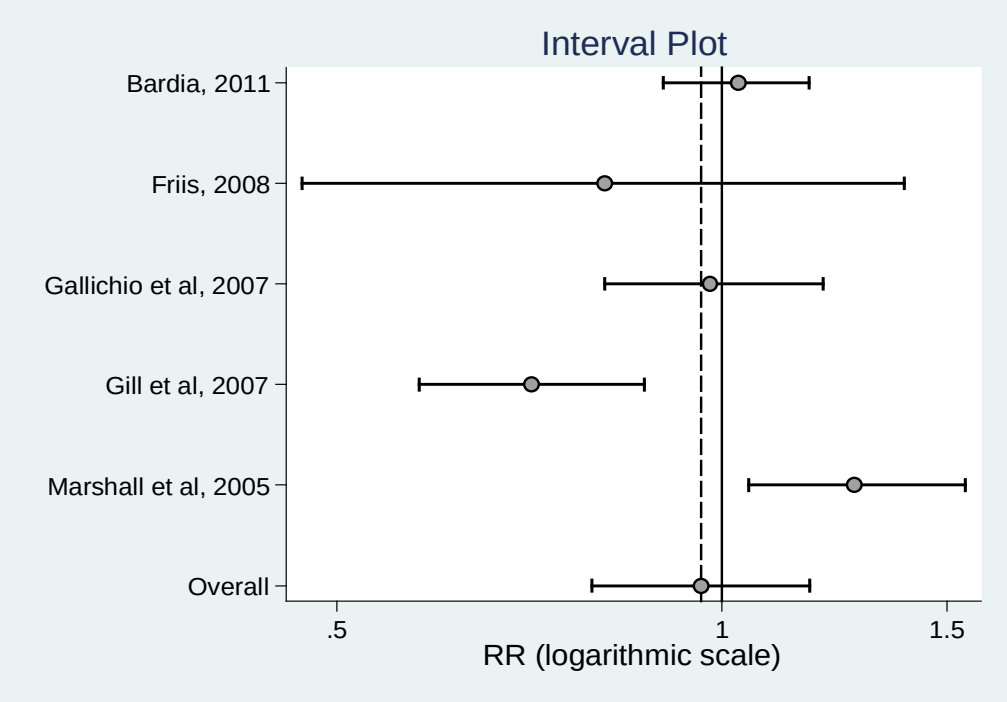
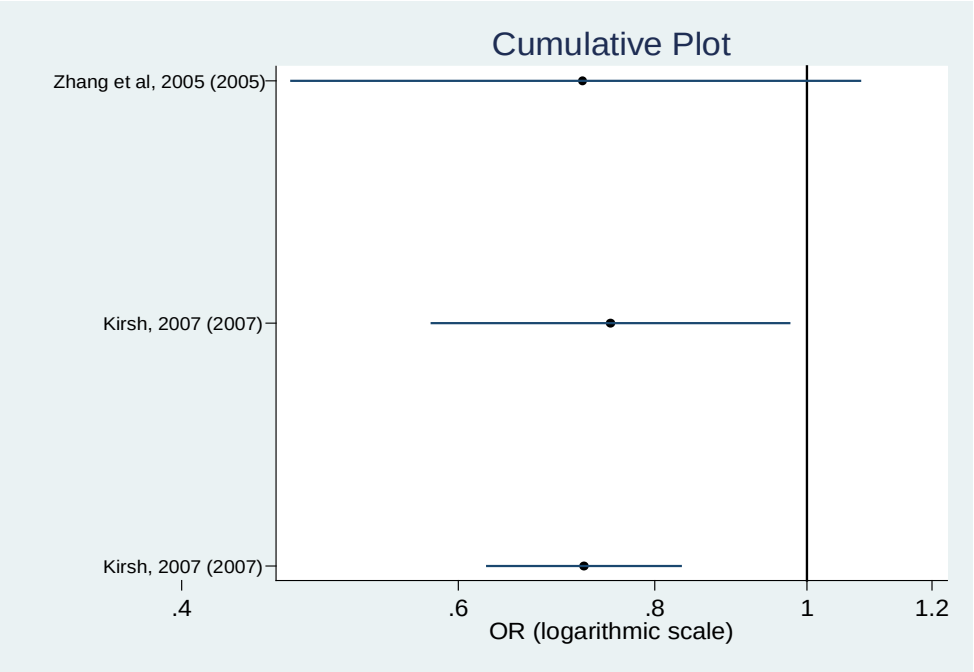


Figure 35. Cumulative meta-analysis forest plot for the relationship between any NSAID and ER+ breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

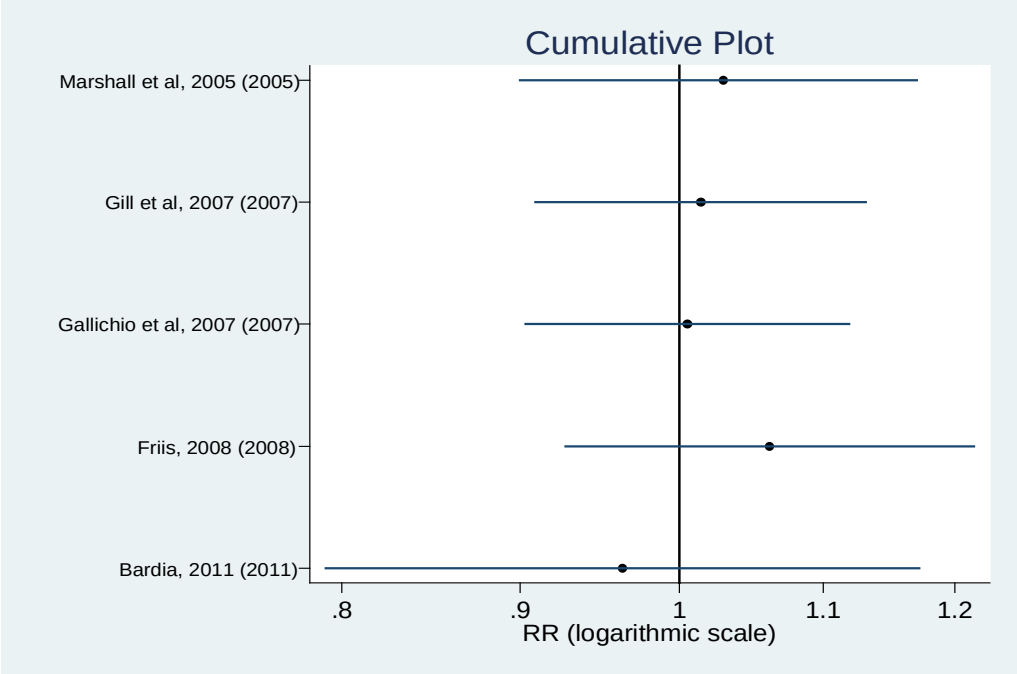
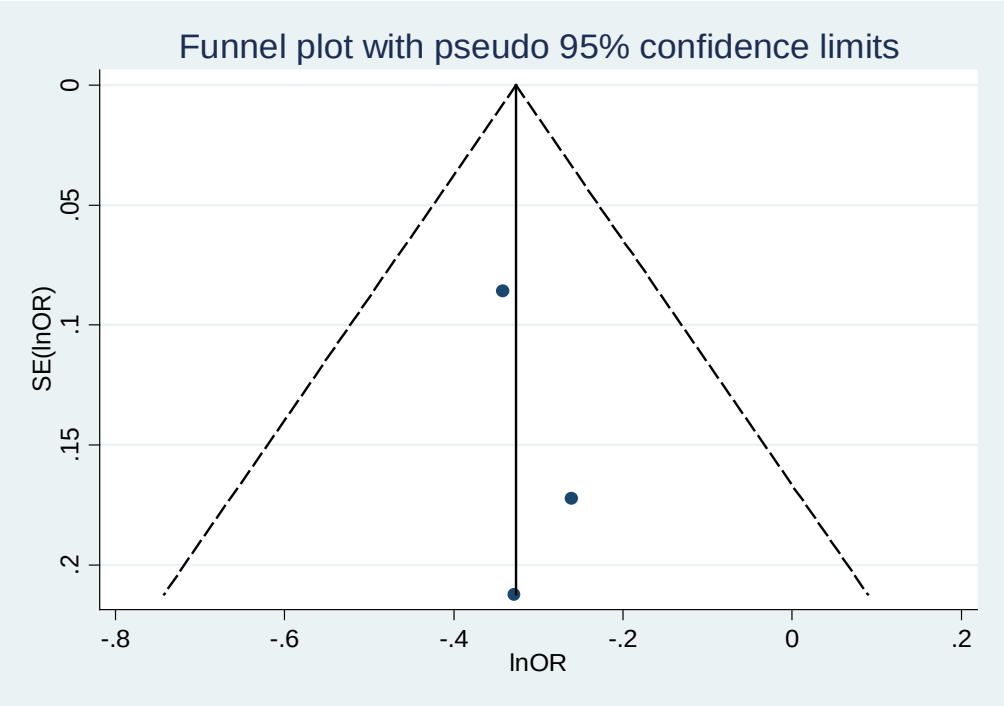


Figure 36. Funnel plot for the relationship between any NSAID and ER+ breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

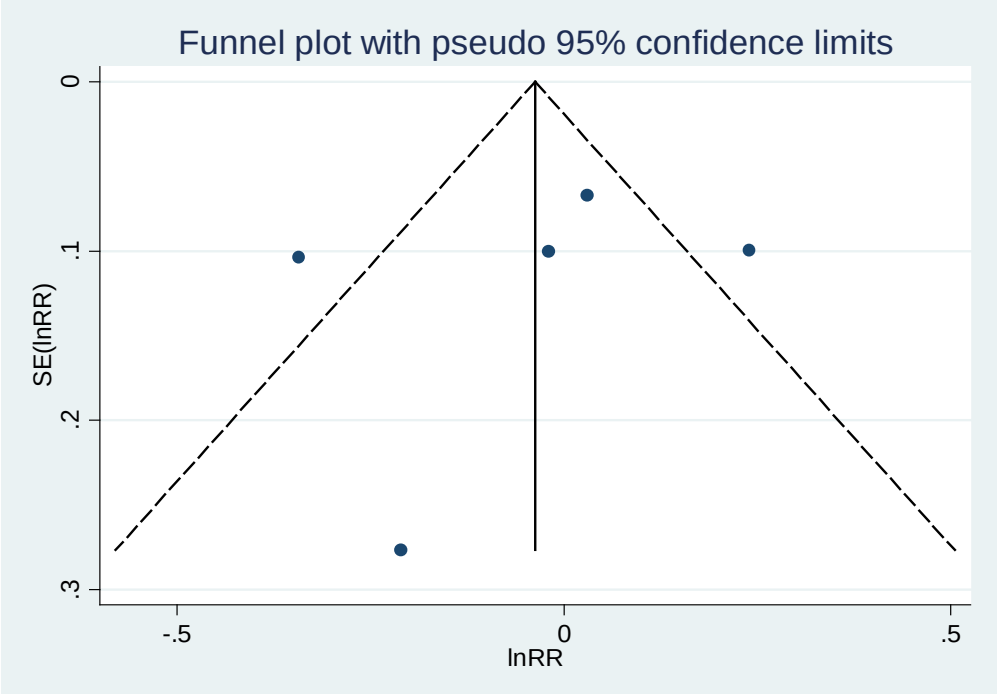
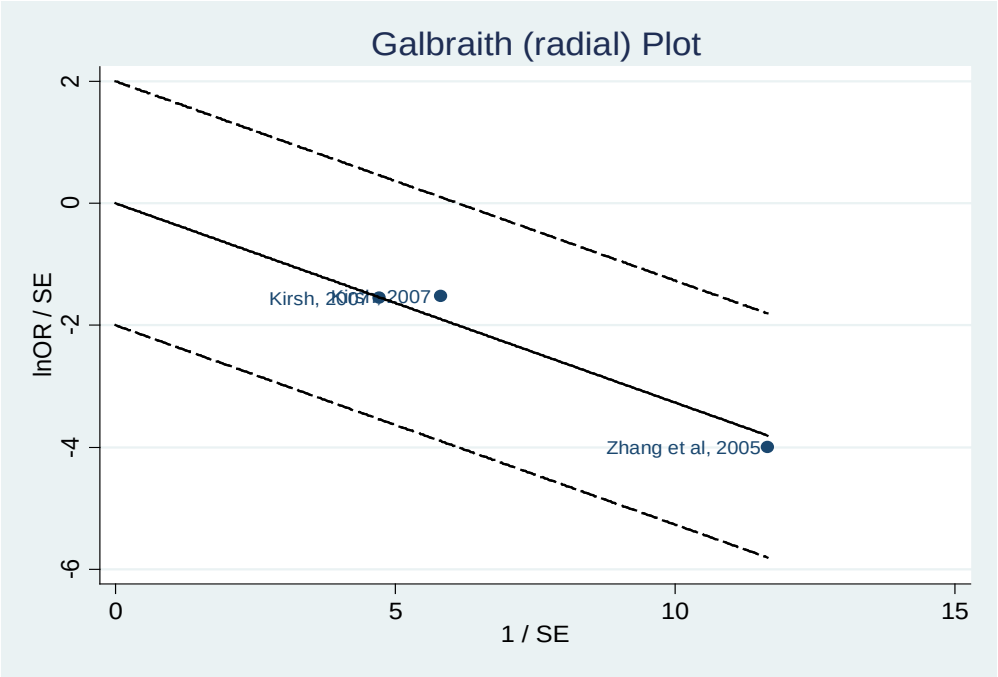


Figure 37. Galbraith radial plot for the relationship between any NSAID and ER+ breast cancer. a) Case-control studies; b) cohort studies.

a)



b)

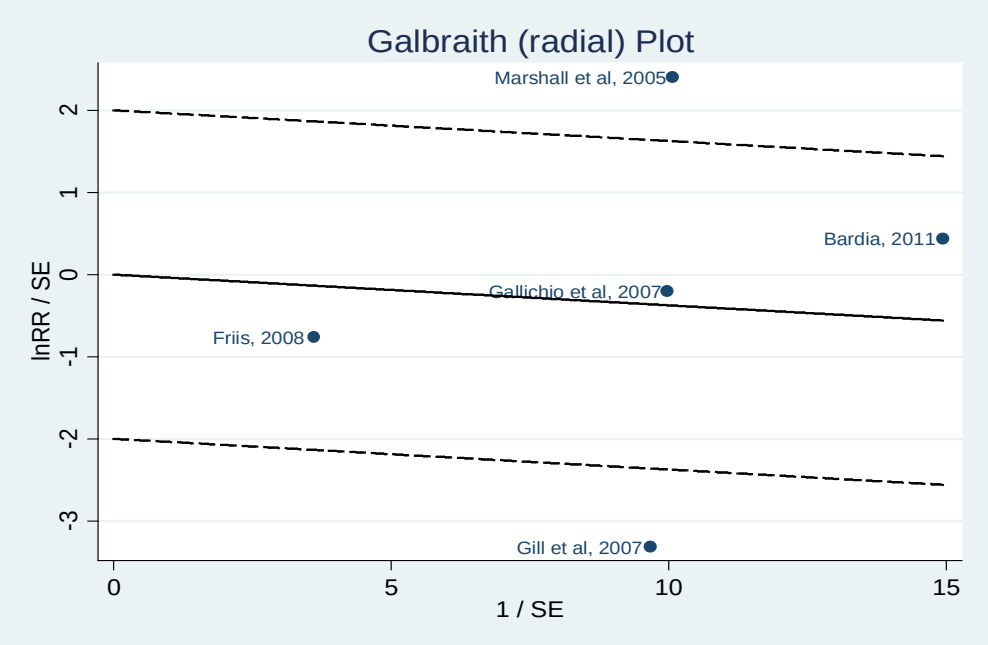
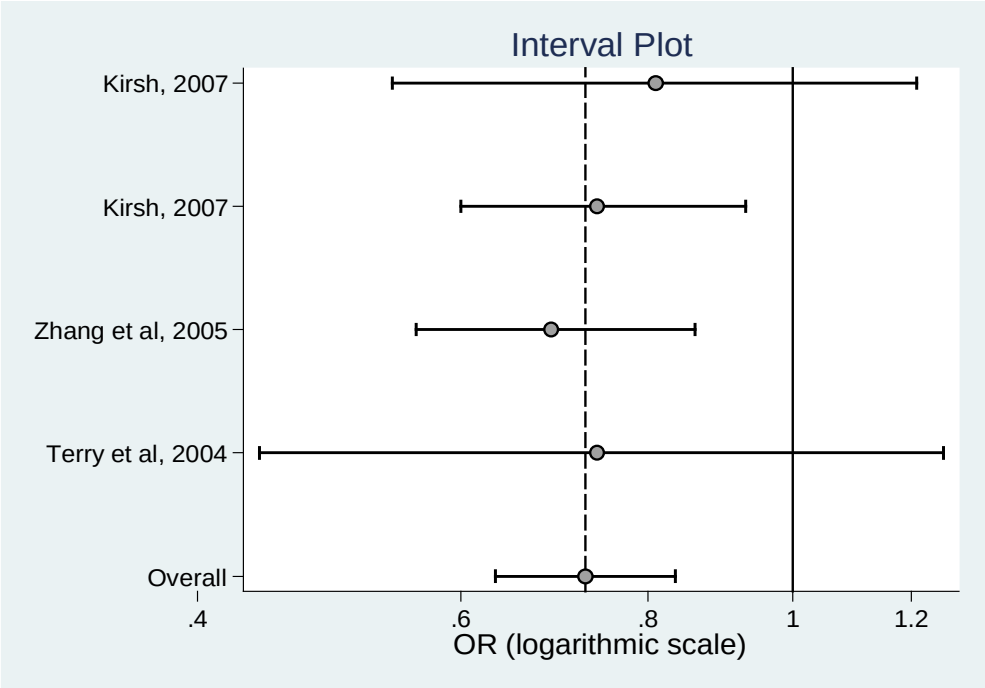


Figure 38. Forest plot for the relationship between aspirin and ER+ breast cancer. a) case-control studies; b) cohort studies.

a)



b)

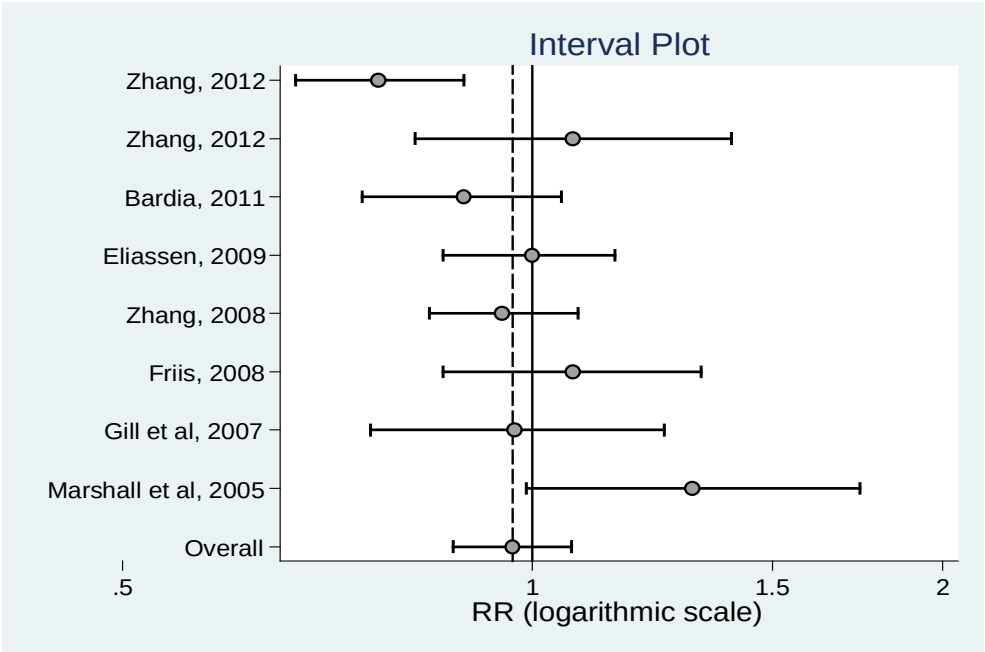
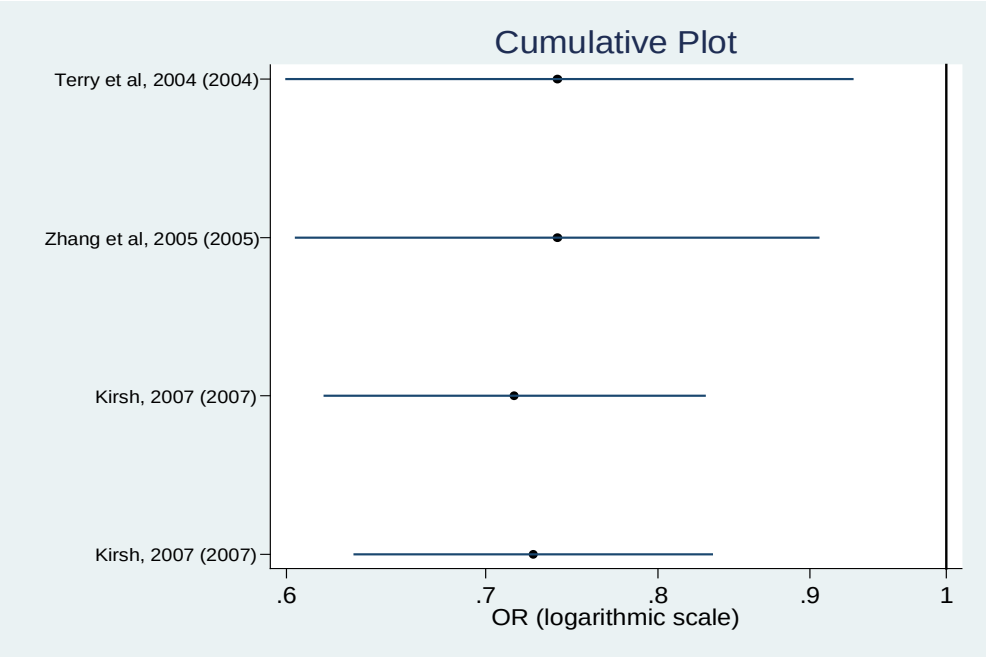


Figure 39. Cumulative meta-analysis forest plot for the relationship between aspirin and ER+ breast cancer. a) case-control studies; b) cohort studies.

a)



b)

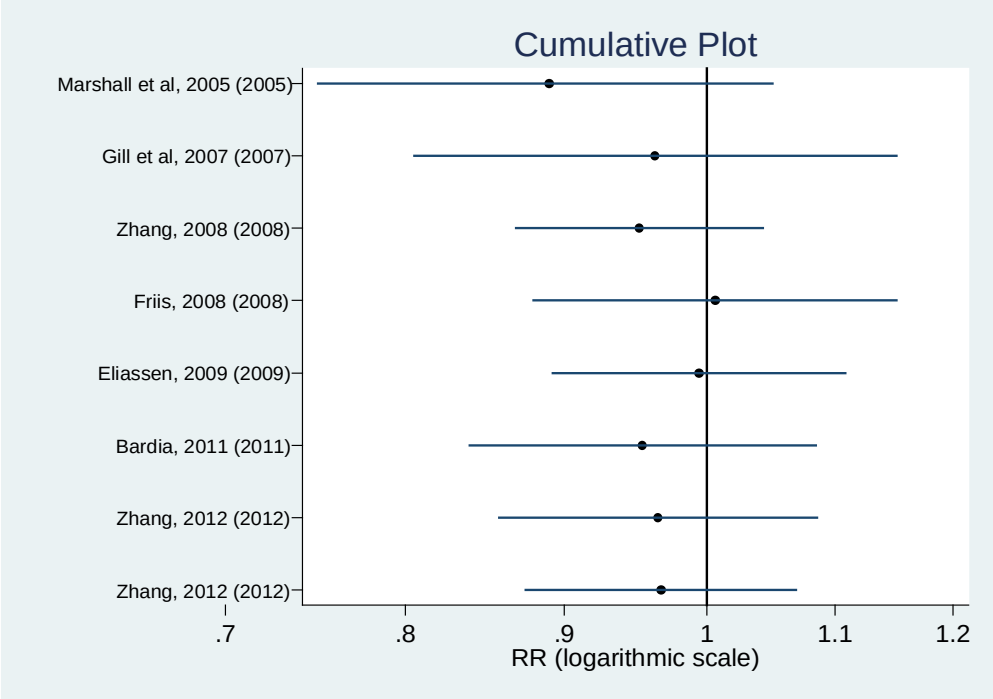
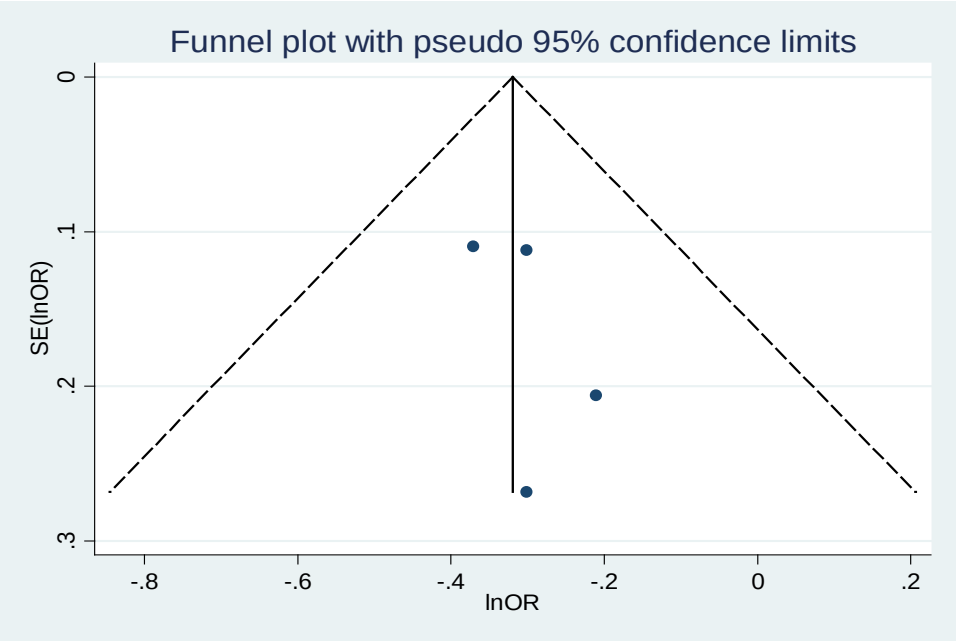


Figure 40. Funnel plot for the relationship between aspirin and ER+ breast cancer. a) case-control studies; b) cohort studies.

a)



b)

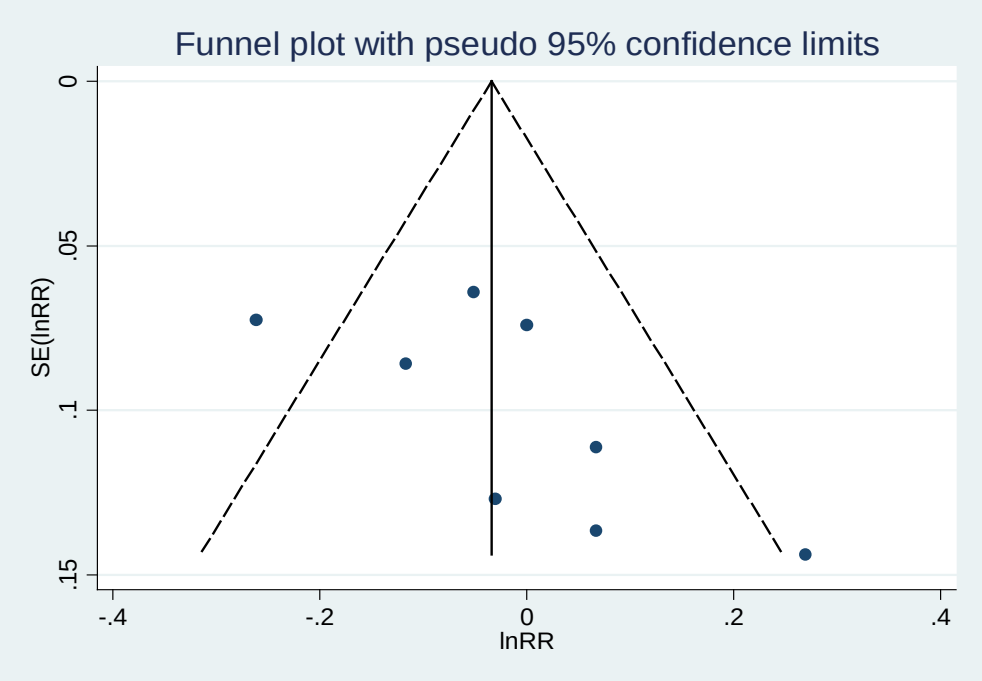
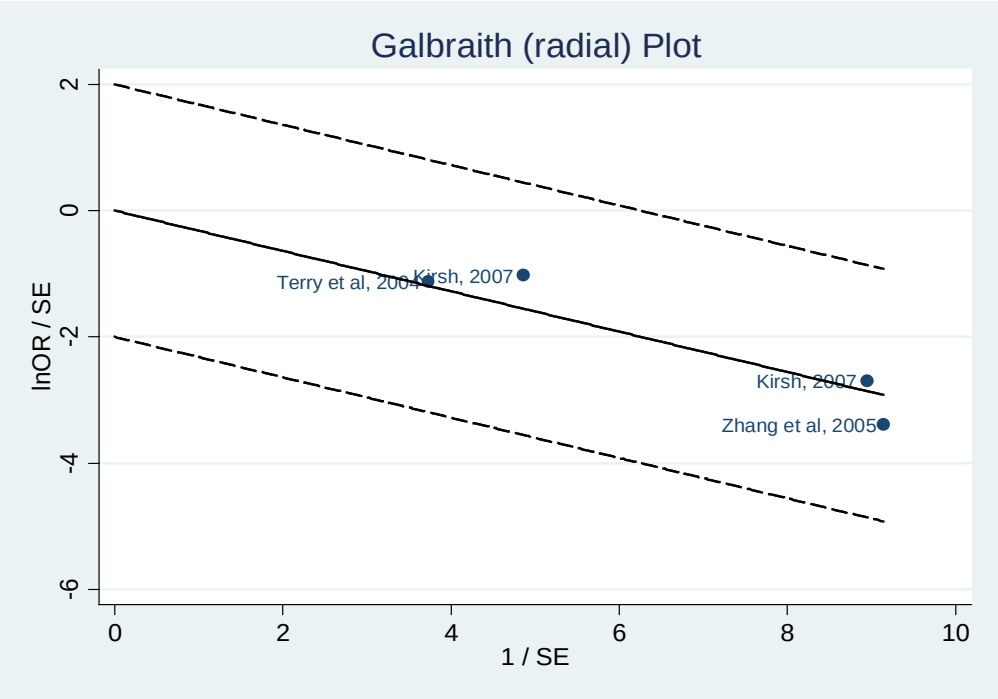


Figure 41. Galbraith radial plot for the relationship between aspirin and ER+ breast cancer. a) case-control studies; b) cohort studies.

a)



b)

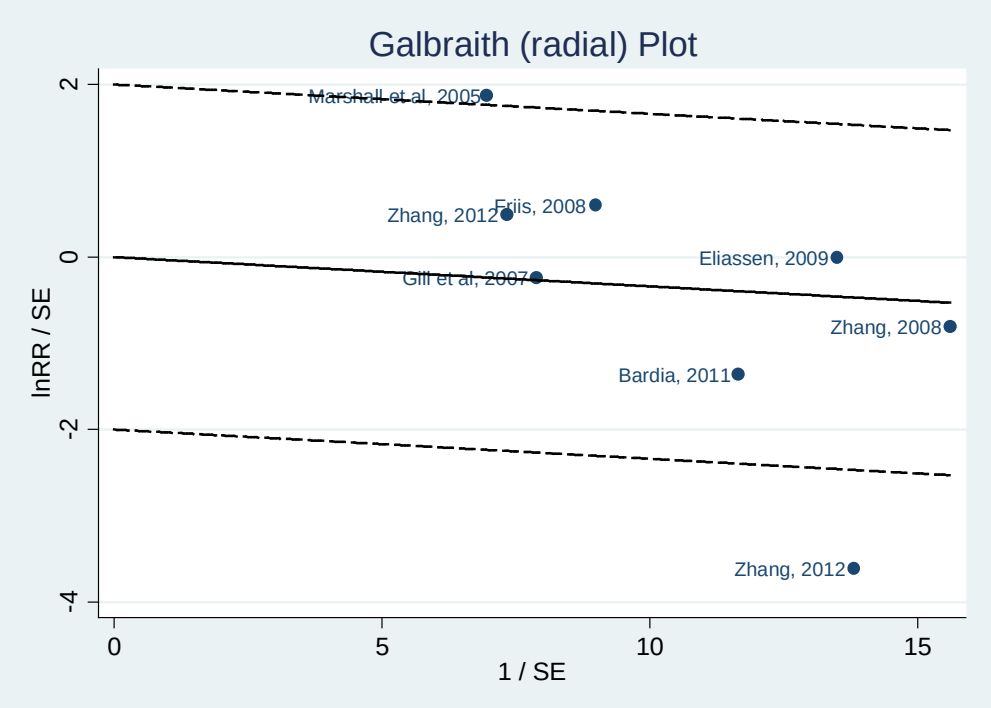


Figure 42. Forest plot for the relationship between acetaminophen and ER+ breast cancer (cohort studies only)

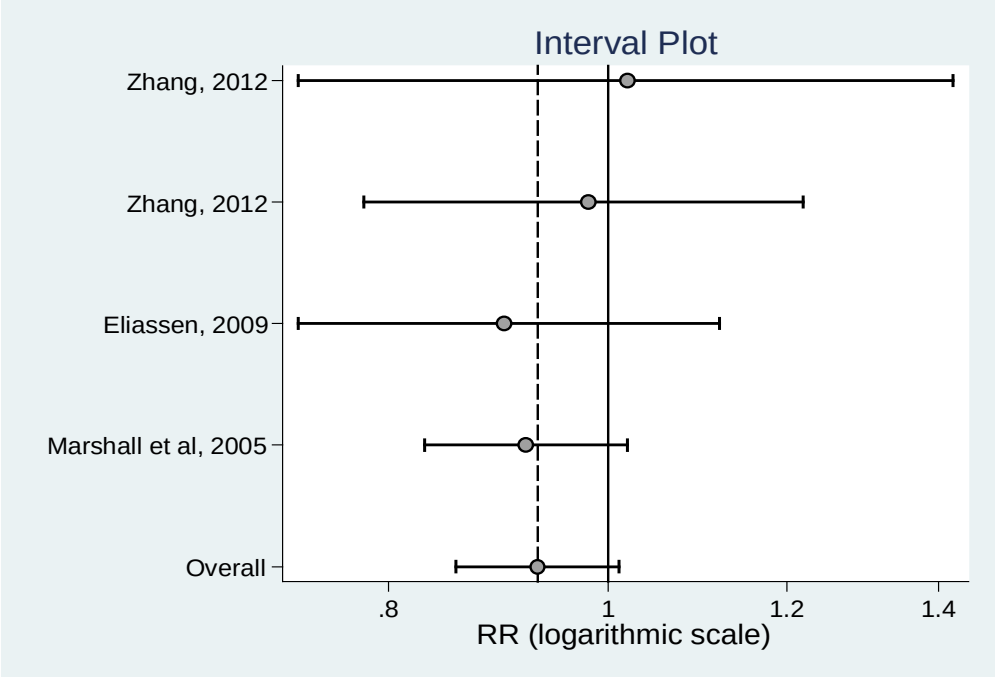


Figure 43. Cumulative meta-analysis fores plot for the relationship between acetaminophen and ER+ breast cancer (cohort studies only)

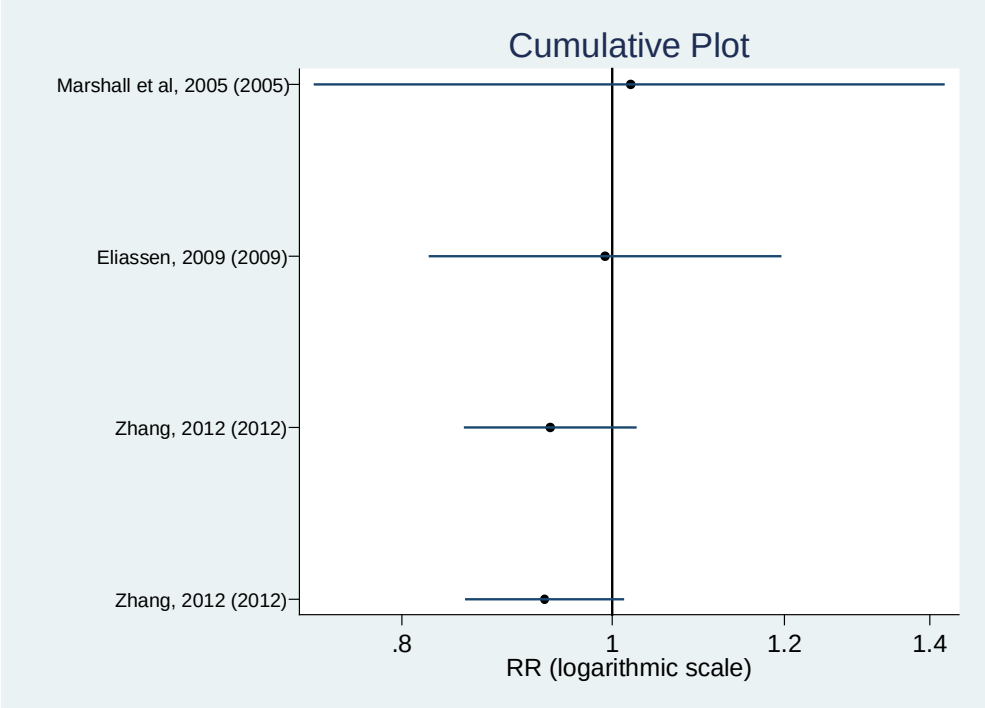


Figure 44. Funnel plot for the relationship between acetaminophen and ER+ breast cancer (cohort studies only)

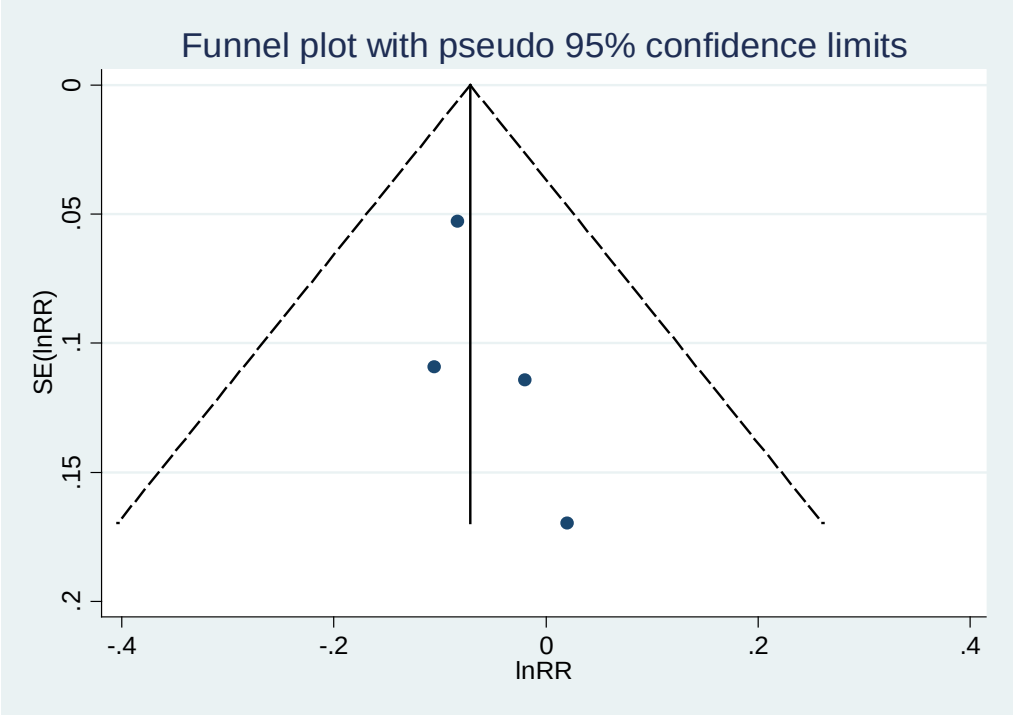


Figure 45. Galbraith radial plot for the relationship between acetaminophen and ER+ breast cancer (cohort studies only)

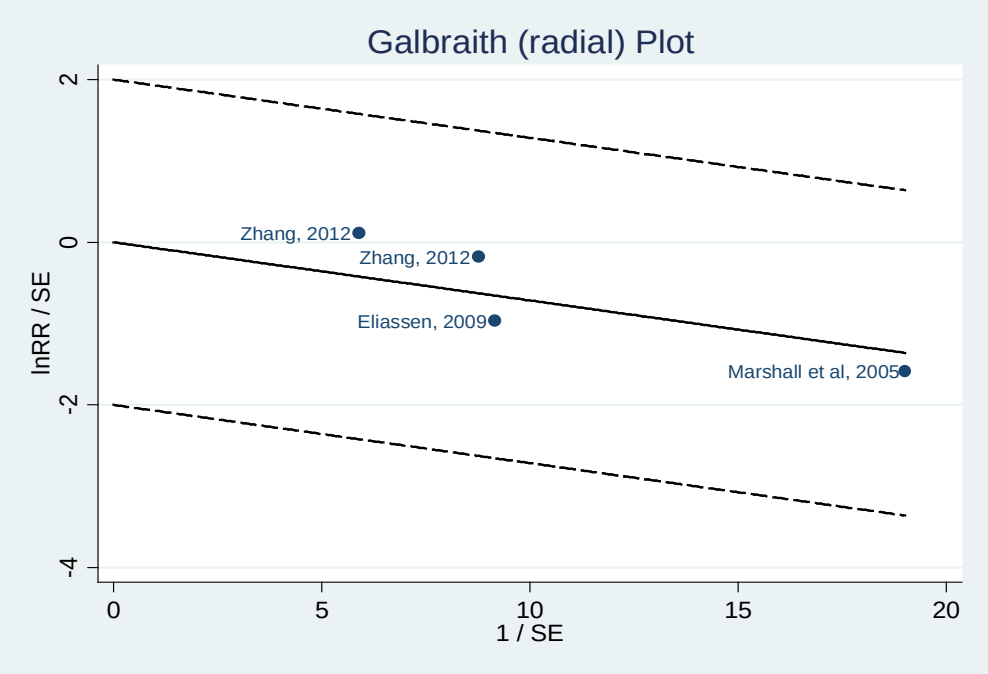


Figure 46. Forest plot for the relationship between non-aspirin NSAIDs and ER+ breast cancer (cohort studies only)

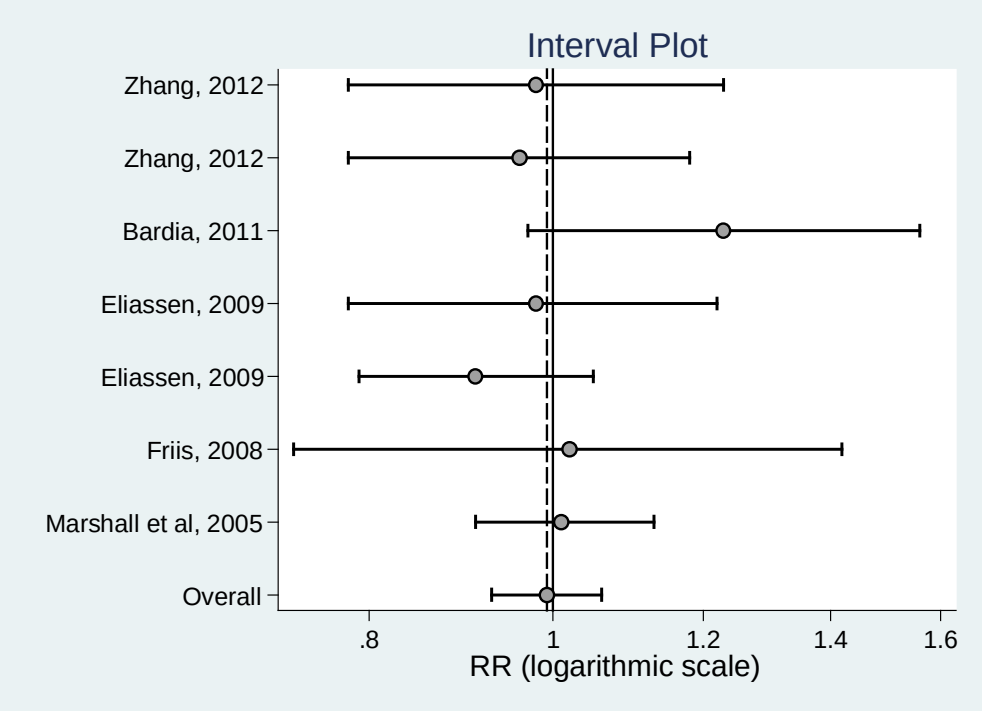


Figure 47. Cumulative meta-analysis forest plot for the relationship between non-aspirin NSAIDs and ER+ breast cancer (cohort studies only)

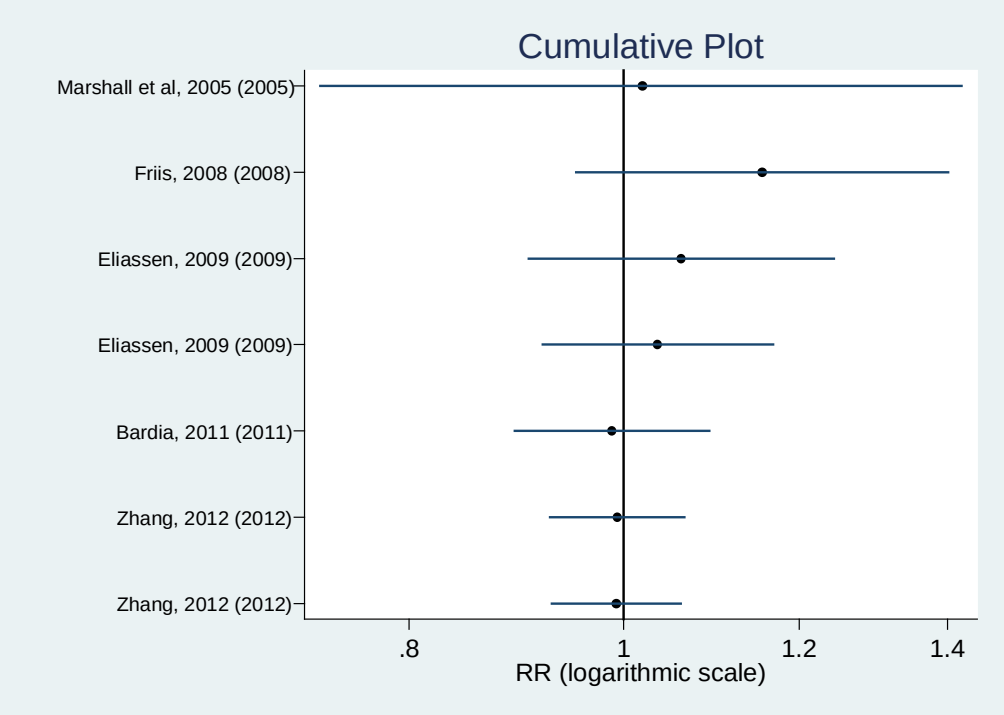


Figure 48. Funnel plot for the relationship between non-aspirin NSAIDs and ER+ breast cancer (cohort studies only)

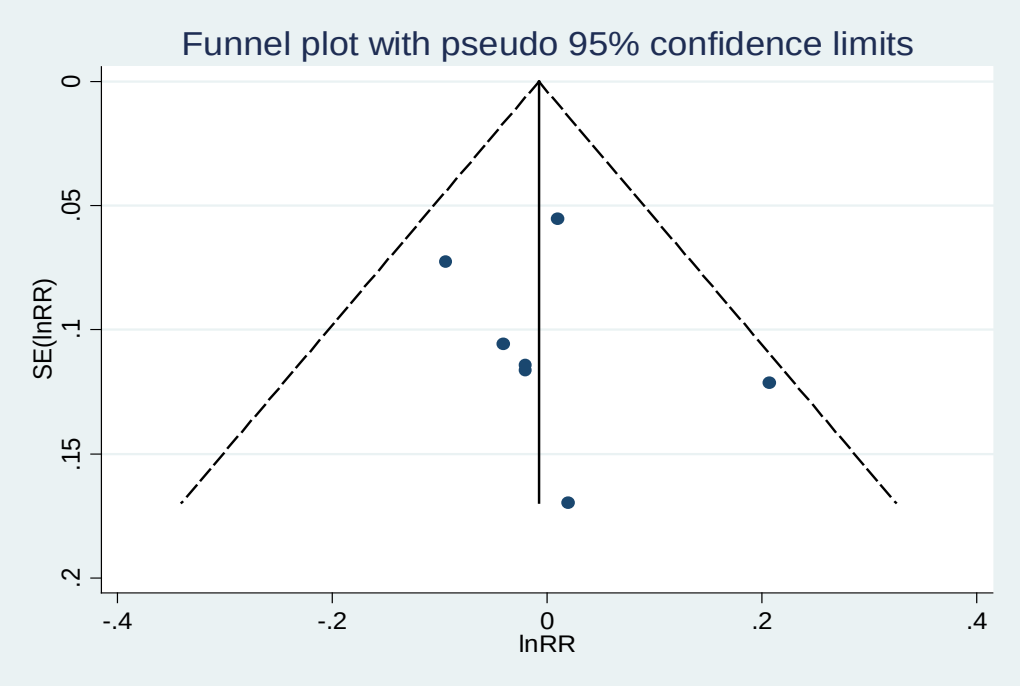


Figure 49. Galbraith radial plot for the relationship between non-aspirin NSAIDs and ER+ breast cancer (cohort studies only)

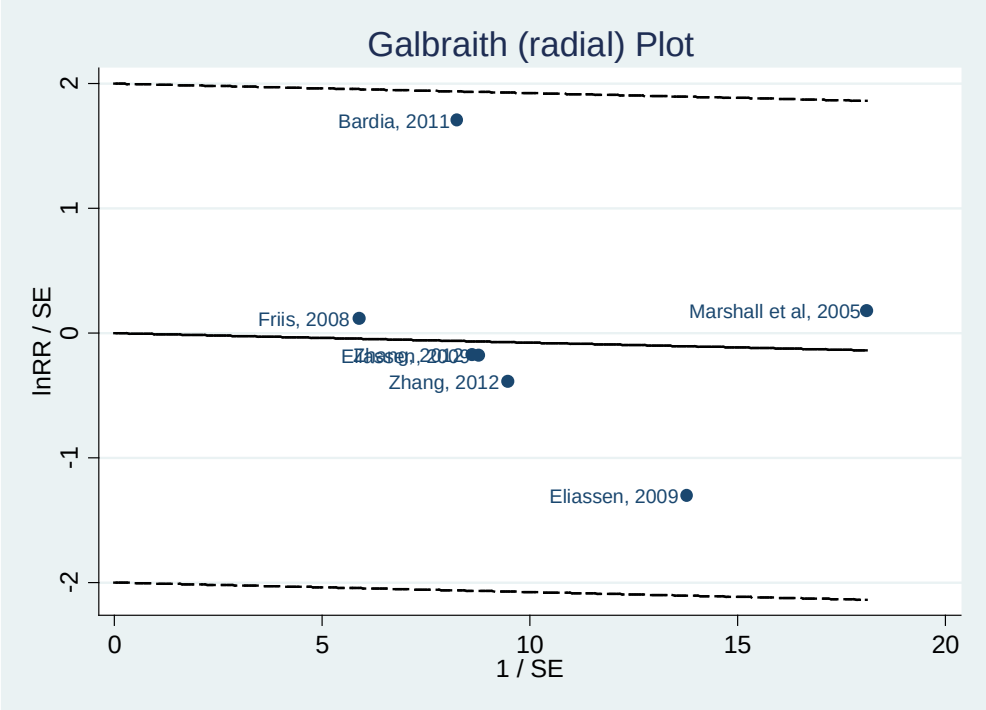
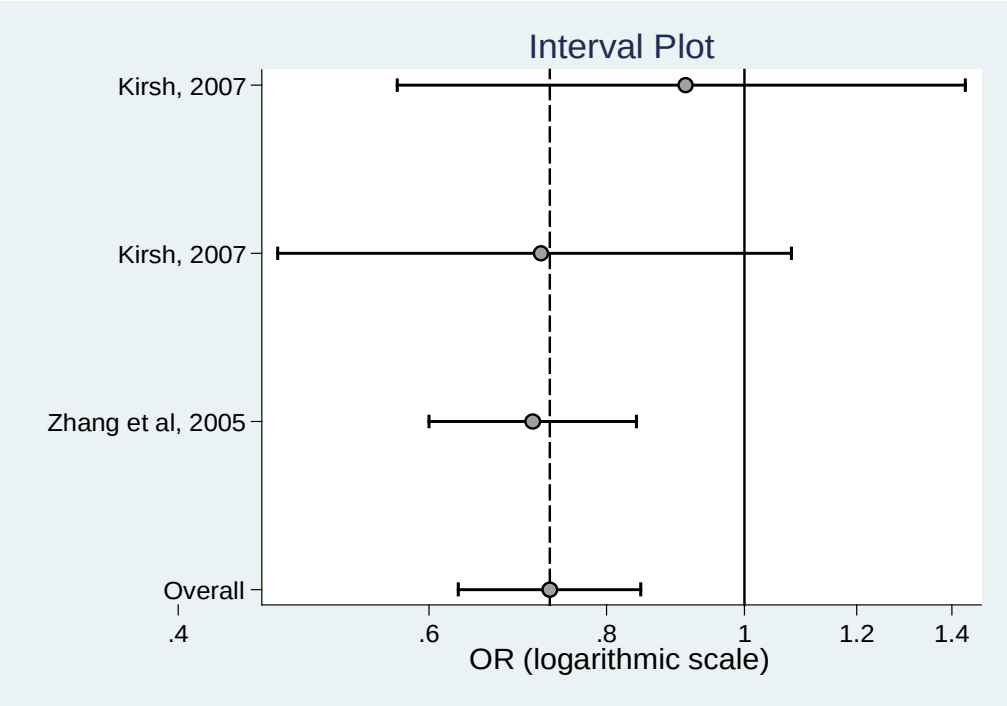


Figure 50. Forest plot for the relationship between any NSAID and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

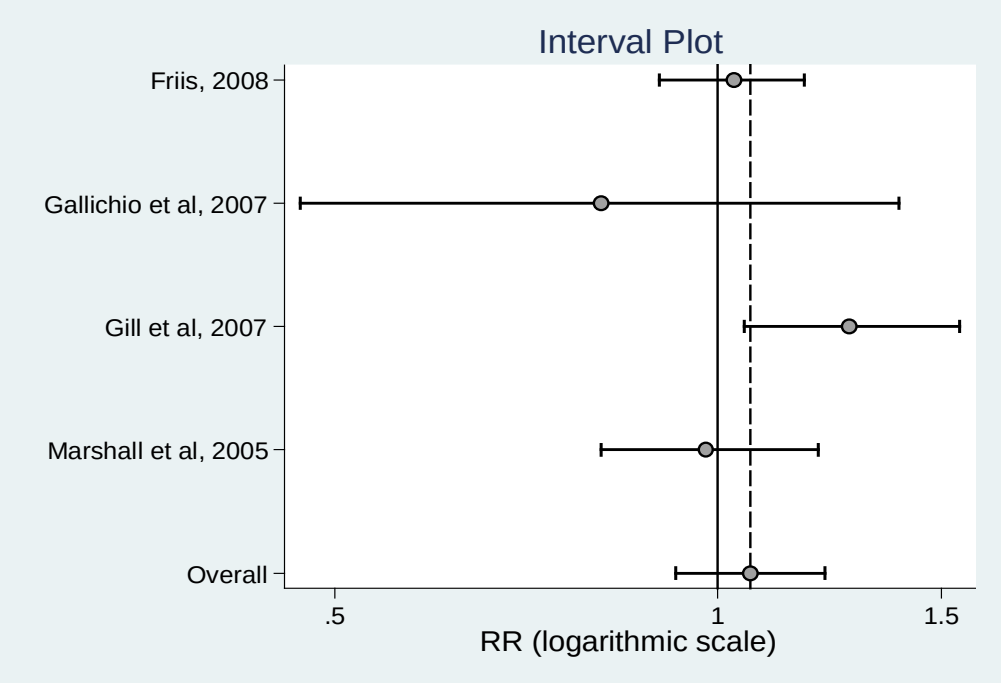
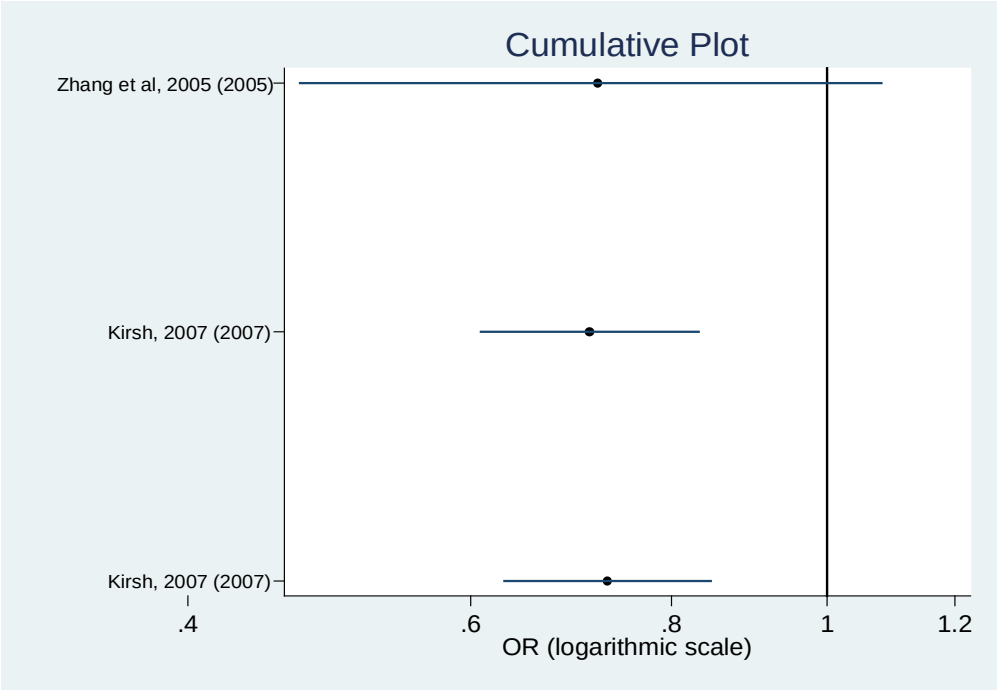


Figure 51. Cumulative meta-analysis forest plot for the relationship between any NSAID and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

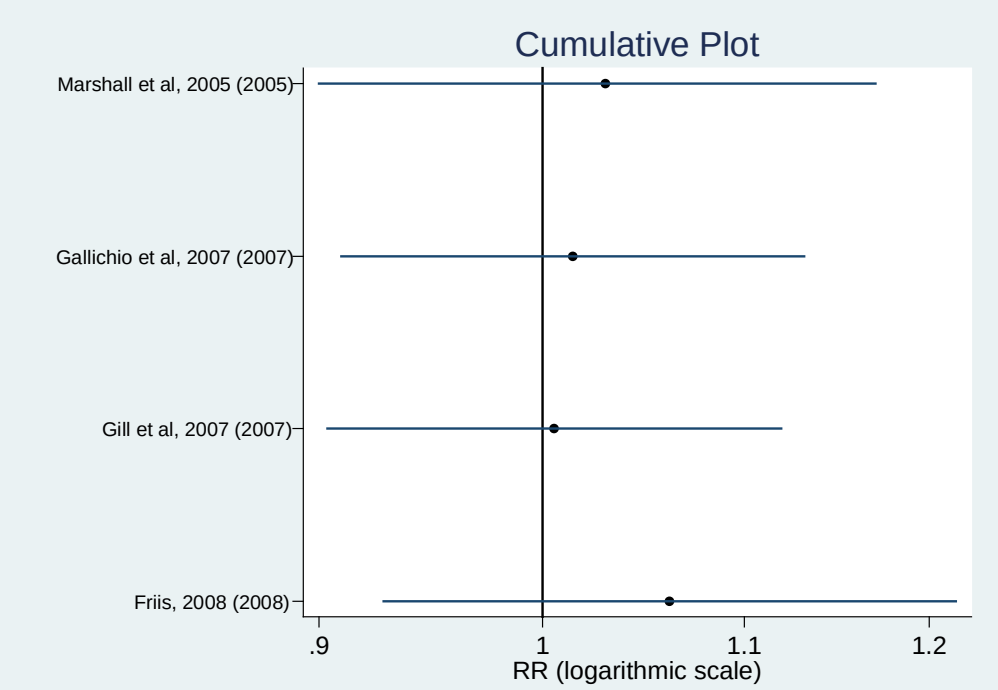
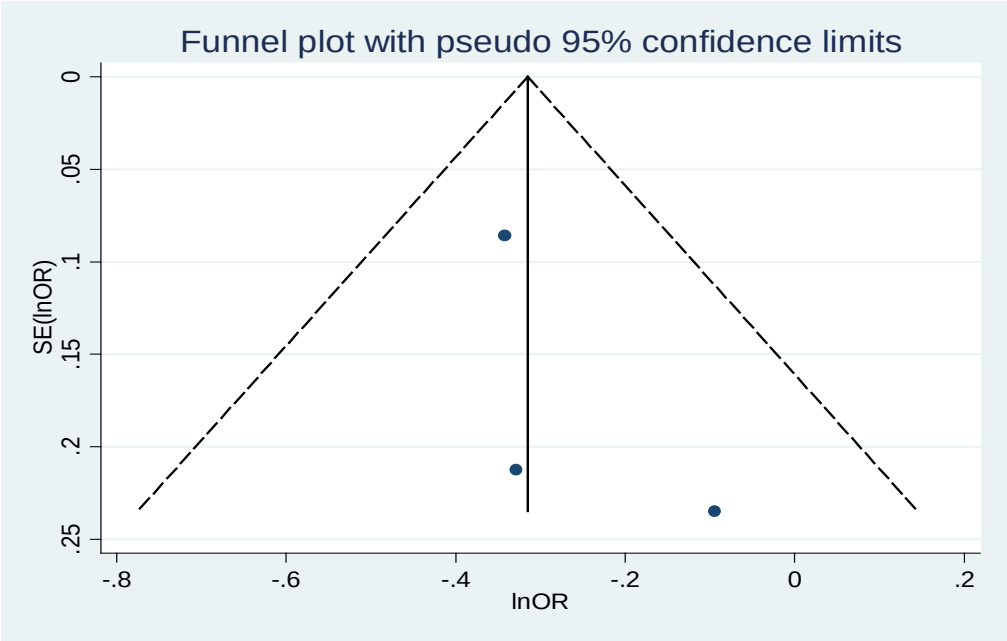


Figure 52. Funnel plot for the relationship between any NSAID and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

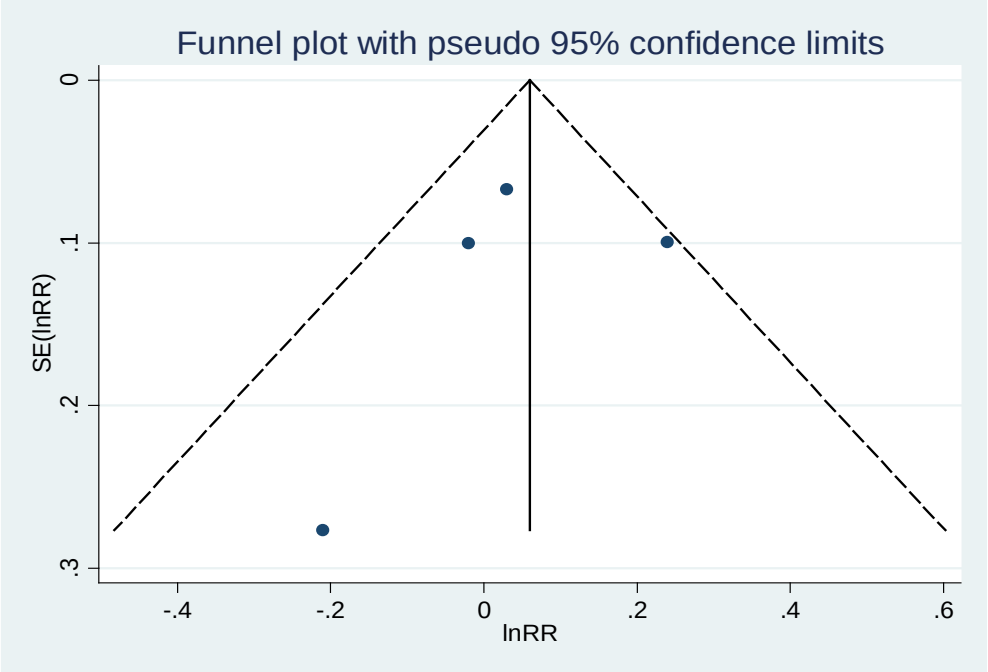
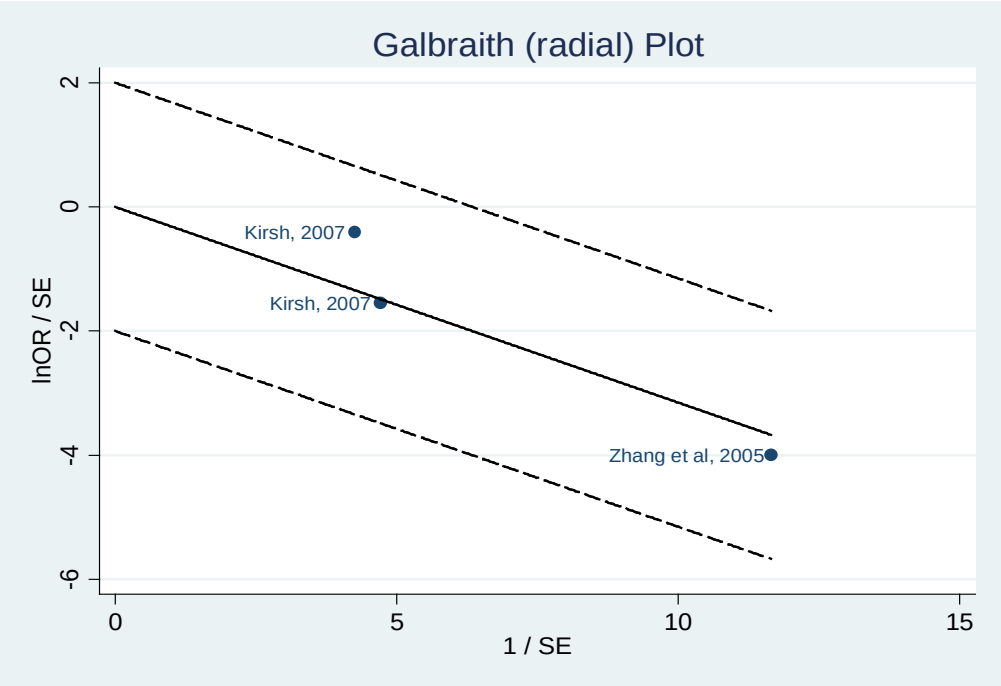


Figure 53. Galbraith radial plot for the relationship between any NSAID and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

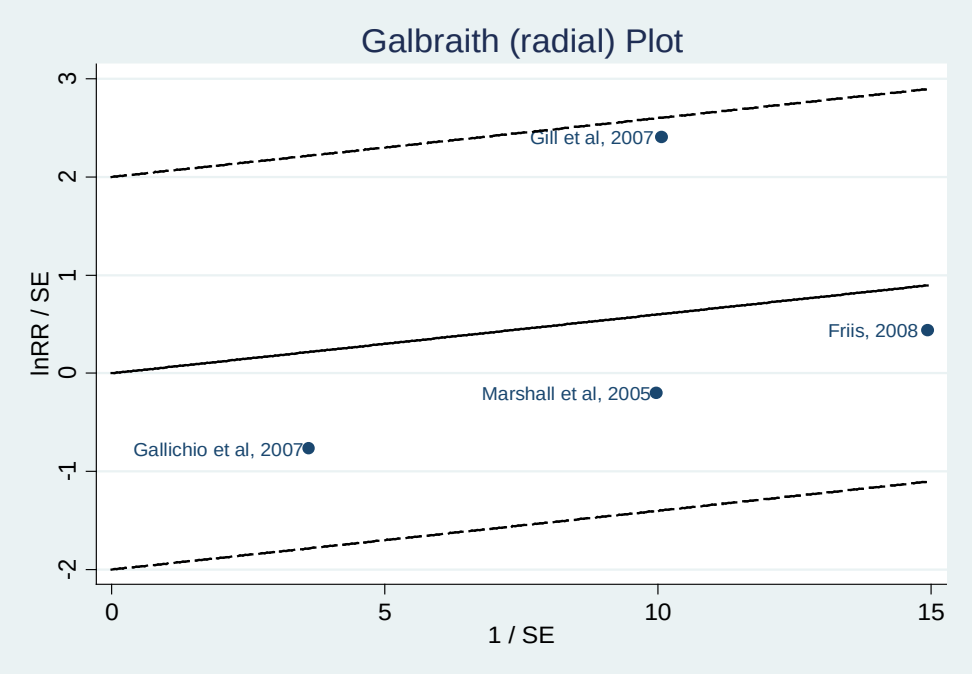
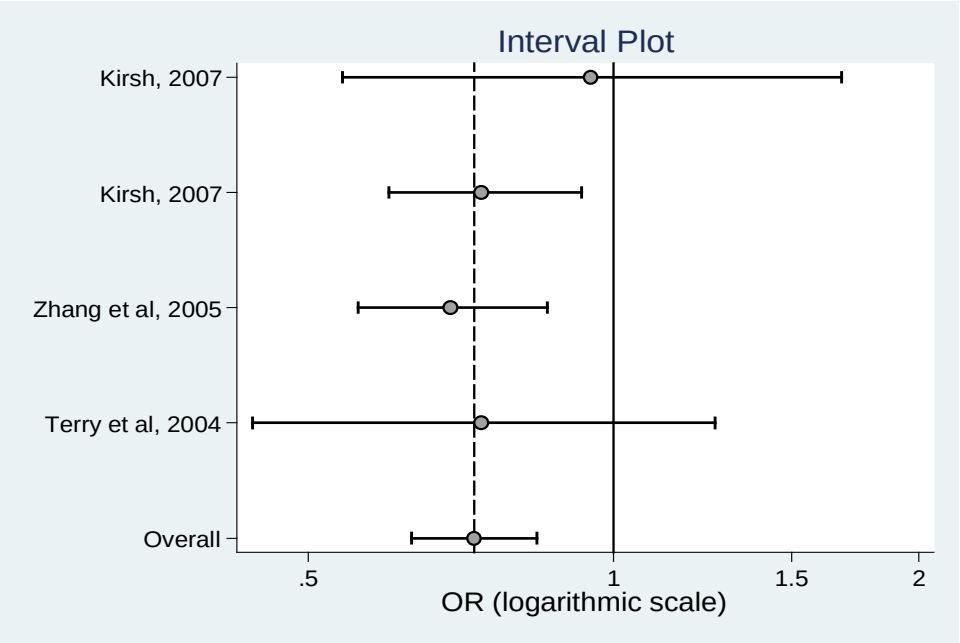


Figure 54. Forest plot for the relationship between aspirin and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

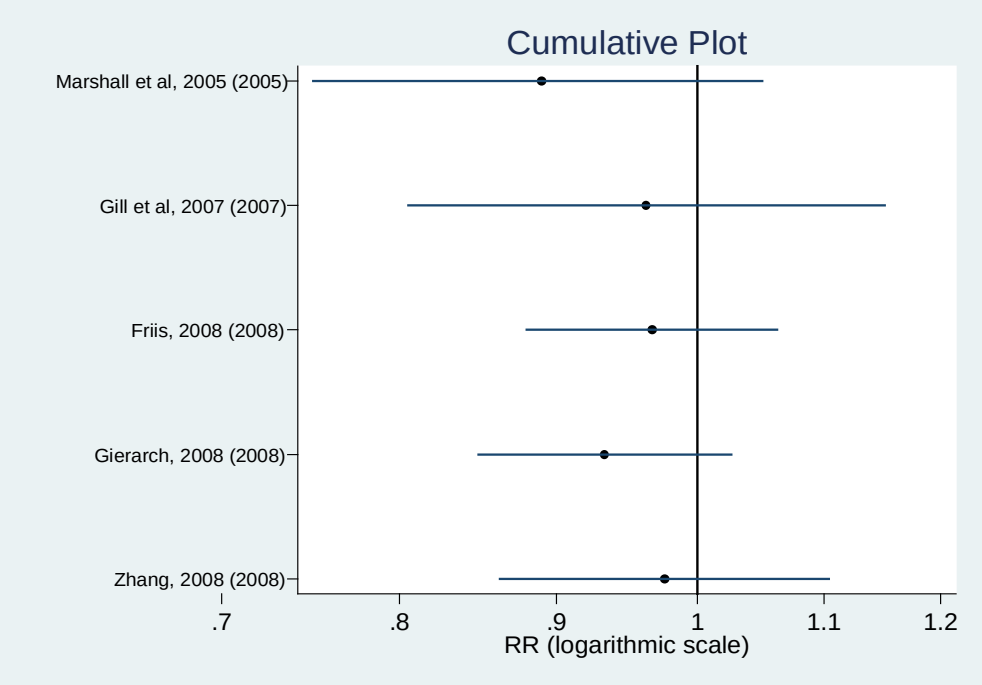
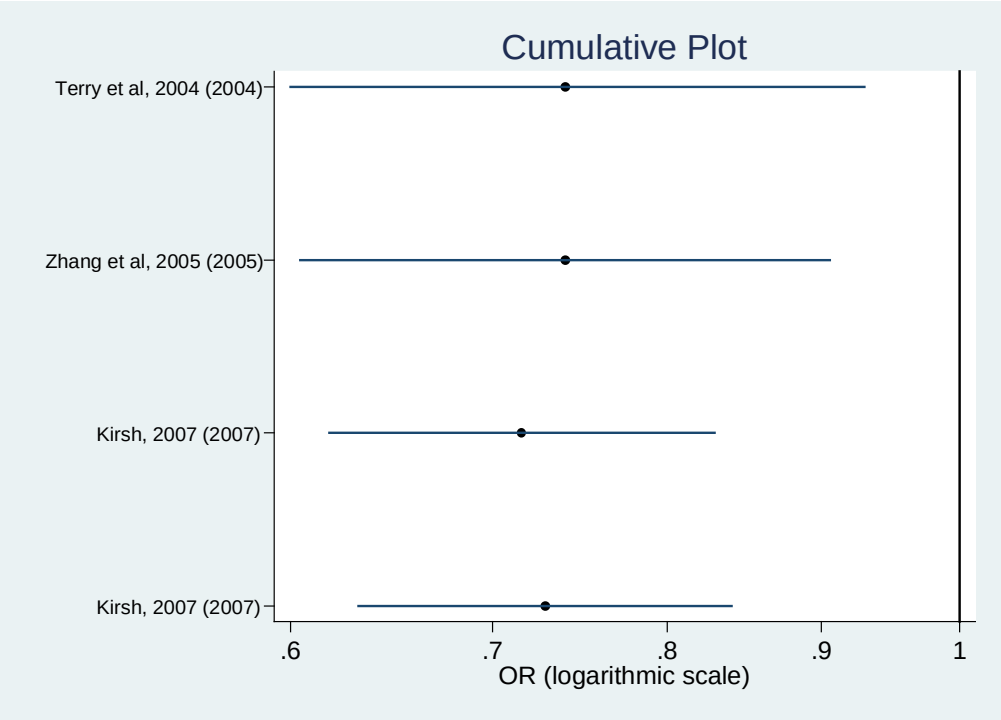


Figure 55. Cumulative meta-analysis forest plot for the relationship between aspirin and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

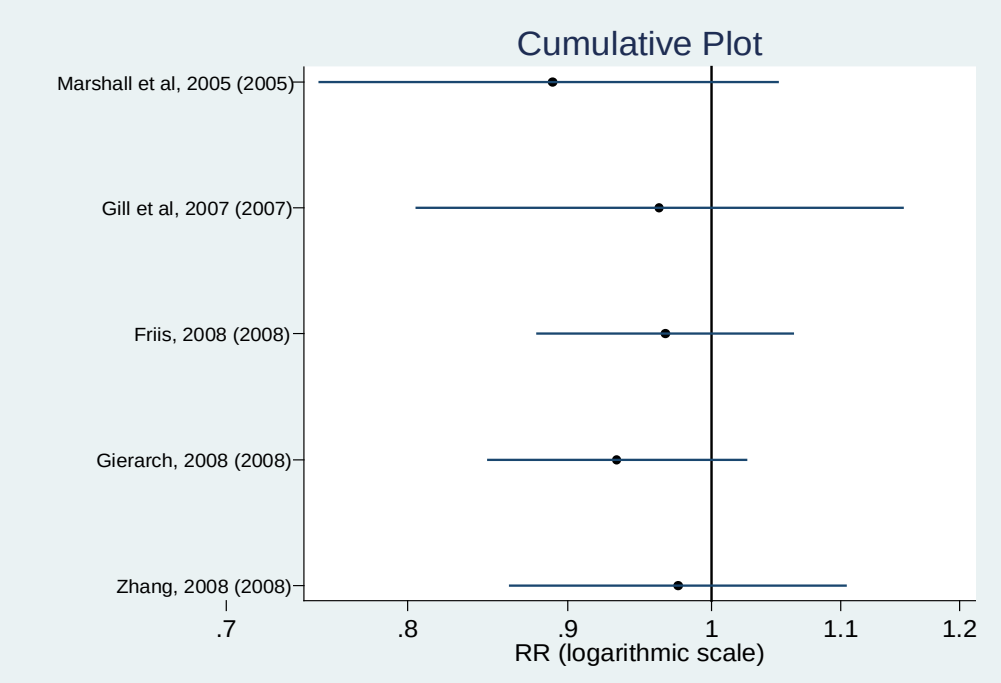
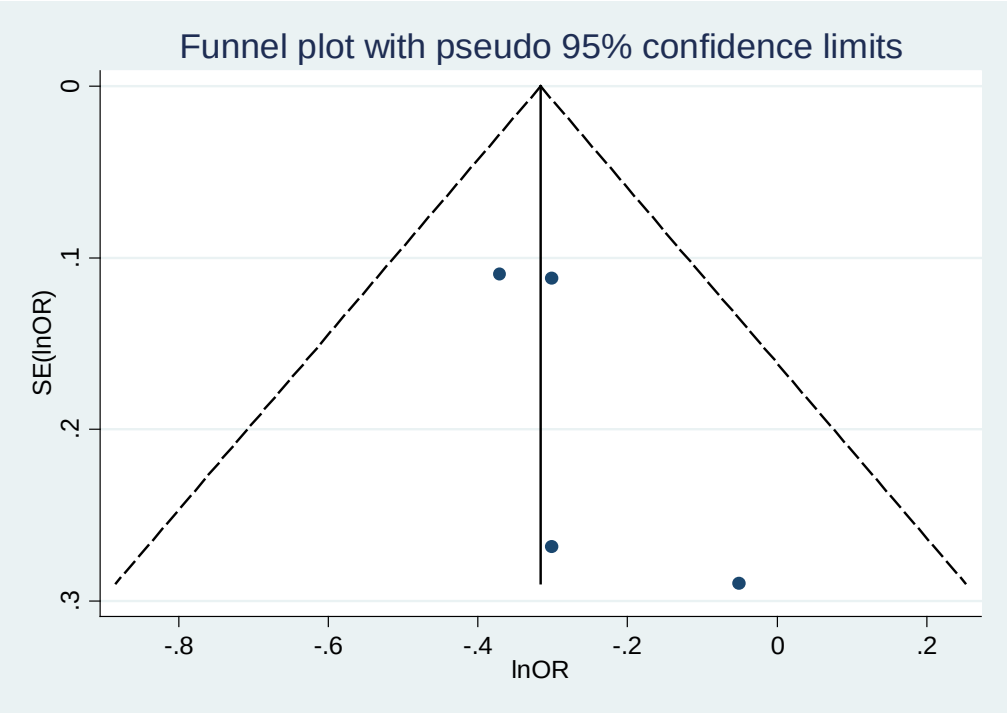


Figure 56. Funnel plot for the relationship between aspirin and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

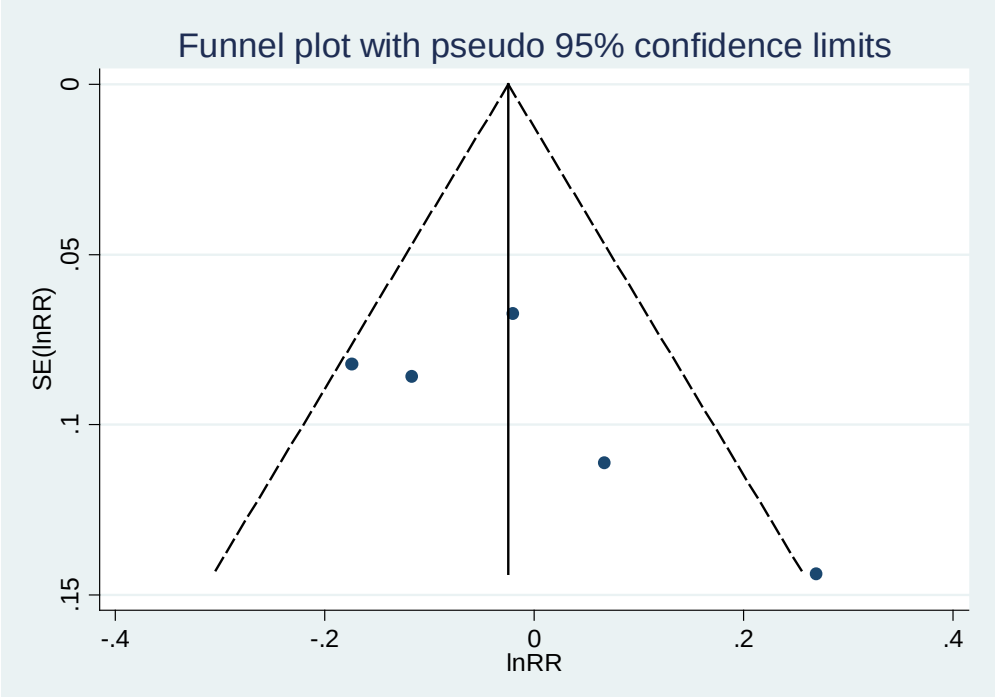
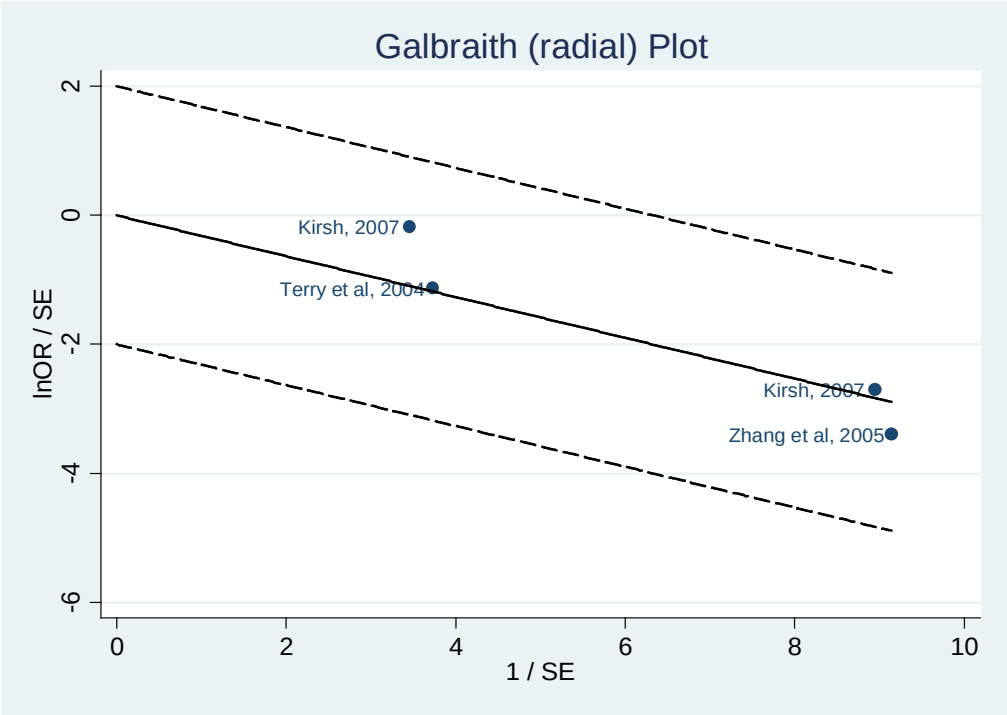


Figure 57. Galbraith radial plot for the relationship between aspirin and PR+ breast cancer. a) Case-control studies; b) cohort studies

a)



b)

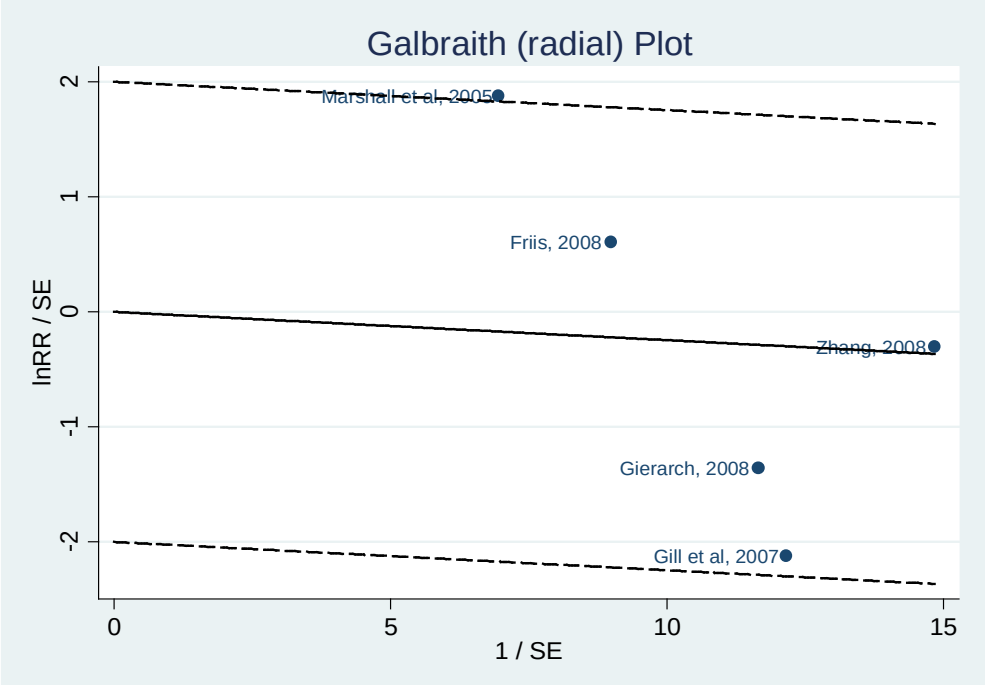


Figure 58. Forest plot for the relationship between non-aspirin NSAIDs and PR+ breast cancer (cohort studies only)

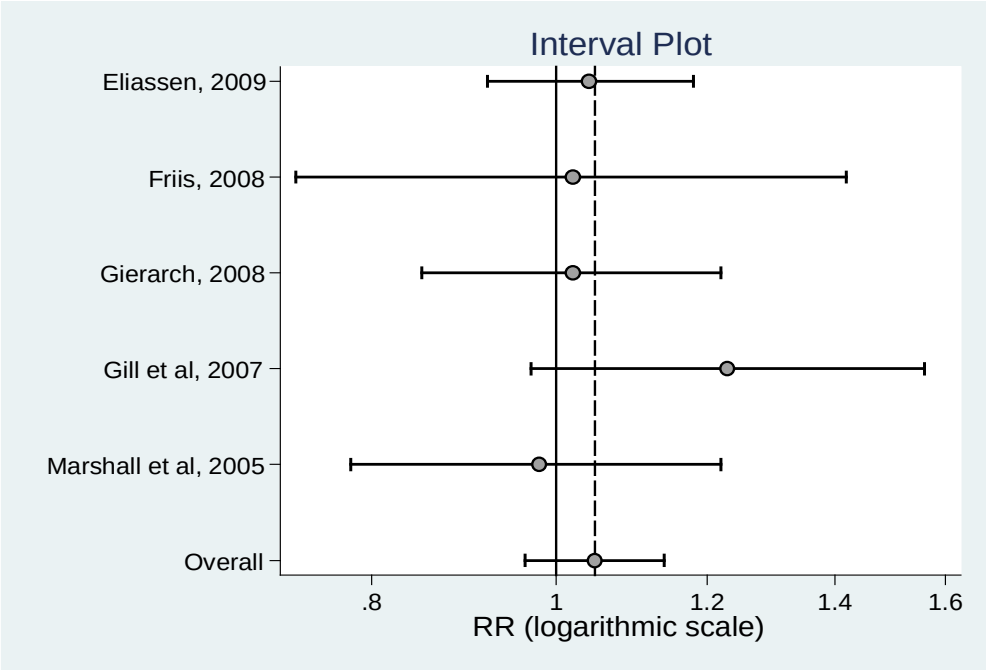


Figure 59. Cumulative meta-analysis forest plot for the relationship between non-aspirin NSAIDs and PR+ breast cancer (cohort studies only)

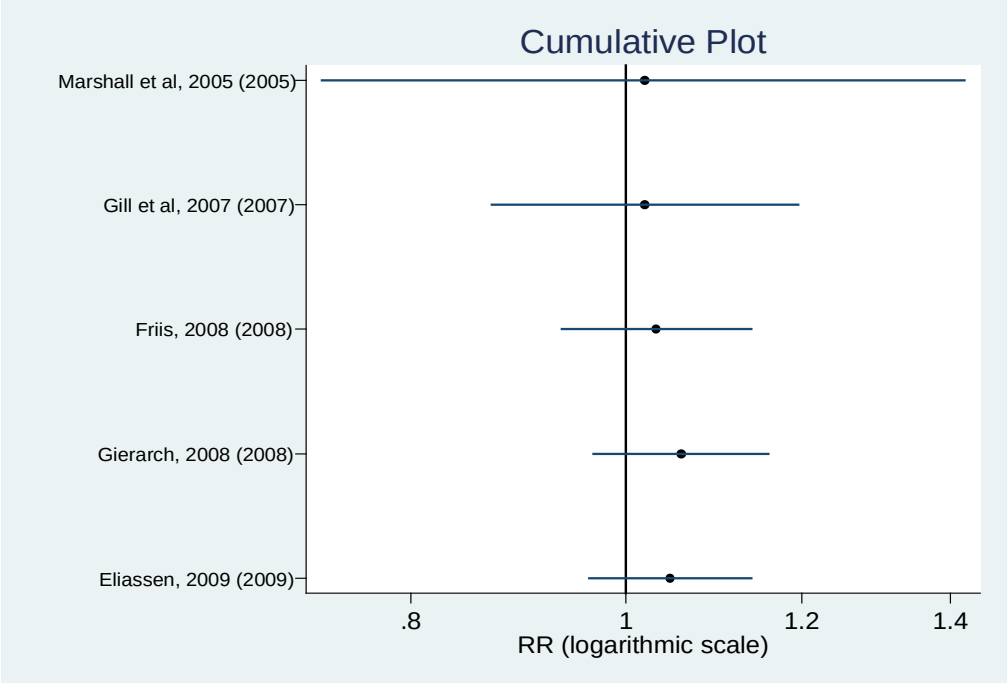


Figure 60. Funnel plot for the relationship between non-aspirin NSAIDs and PR+ breast cancer (cohort studies only)

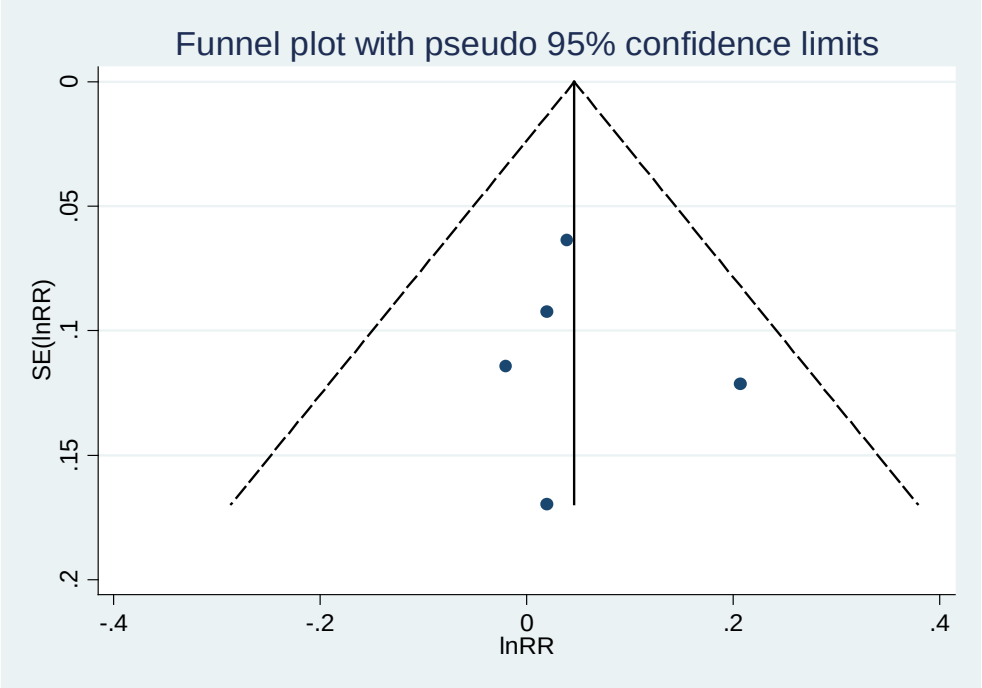
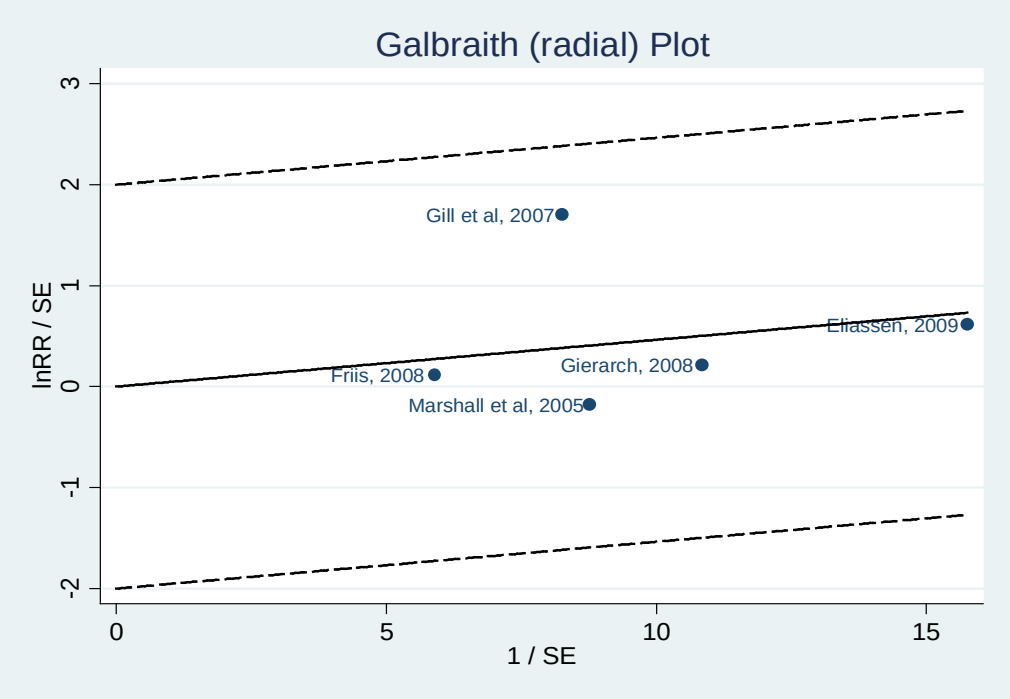


Figure 61. Galbraith radial plot for the relationship between non-aspirin NSAIDs and PR+ breast cancer (cohort studies only)



DISCUSSION

Breast cancer is the most frequently diagnosed neoplasia globally and the first cause of death from cancer in women (Chen, 2014). Its 5-year survival rate, however, is one of the highest among all cancers (89.2% in the US, 82% in Spain) (Howlander *et al.,* 2014; Ferlay *et al.,* 2015). Therefore, this is a major epidemiological problem, which largely justifies the amount and quality of resources it has been receiving for decades.

Age is the most important risk factor for breast cancer, to the extent that premenopausal and postmenopausal breast tumors can be considered as different entities based on their hormonal, molecular and histological features.

Multiple criteria have historically been used, based on such features, to classify breast cancer. For decades, the main parameters have been clinical (age, tumor size, node involvement, presence of distant metastases and histological grade) and pathological (estrogen receptor [ER], progesterone receptor [PR] and human epidermal growth factor receptor 2 [HER2], and they are still used in the clinic in order to assess the disease prognosis and decide treatment plans. For instance, the expression of estrogen receptors in a breast tumor (termed "estrogen receptor positive" or "ER+") suggests that its growth is influenced by estrogen, among other factors, and it is susceptible for antiestrogen treatment. On the other hand, tumors that do not express such receptors ("estrogen receptor negative" or "ER-") depend on factors other than estrogen for their progression and they are, therefore, not amenable to hormonal treatment. Two out of three breast cancers express some type of hormonal receptor.

Similarly to most diseases, both environmental and genetic of factors must be jointly taken into account in order to understand how breast cancer develops. Most women (80-85%) will need to acquire multiple mutations, which will accumulate during their lifetime before cancer appears. A smaller group of women (15-20%) will already be born with some of those mutations and they will not need so many additional mutations in order for the disease to develop, which will usually occurs earlier than in the first group (Isaacs *et al.,* 2012).

Therefore, globally considered, 80 to 85% breast cancer cases could be considered sporadic, i.e., with no family history and mainly determined by environmental factors, whereas the remaining 15-20% corresponds to hereditary forms of cancer, presenting family history of breast cancer in first-degree relatives, whether the implicated germ-line mutations leading to cancer are known or not.

Among the environmental factors, the most relevant for developing breast cancer are those included in the Gail Model, as previously explained: age at menarche, age at first birth and number of breast biopsies. Additionally, age at menopause, parity and age at first birth, breastfeeding, miscarriage and induced abortion and hormone levels (either endogenous or exogenous) have demonstrated their influence on the individual breast cancer risk. Finally, there is a less relevant set of factors, mostly related to lifestyle and diet, with a weaker involvement in the disease and which are often surrogate indicators of hormonal and reproductive factors. Particularly, exposure to different compounds and drugs -except for hormone replacement therapy- has been widely but irregularly studied, with uneven results. The table below (Table 19) summarizes risk and protective factors for breast cancer, classified by strength of association and magnitude of effect.

Even though the most relevant environmental factors are hormonal and reproductive, inflammation has proved its role in breast carcinogenesis, especially through the COX/PG pathway.

Molecular studies have demonstrated that overexpression of COX-2 is a key feature of all stages of breast cancer. Furthermore, COX-2 is commonly found in premalignant lesions (dysplasia and atypia), carcinoma *in situ*, invasive cancer, and in particular, metastatic disease. In stark contrast to mammary cell populations that are found in different stages of carcinogenesis, COX-2 is usually not detectable in normal (non-inflammed) mammary tissues (Wu, 1996; Dubois *et al*., 1998). Interestingly, some studies also suggest an association between *COX-2* expression and aggressiveness criteria, such as large tumor size, low grade of differentiation, high proliferation rate, metastasis formation, absence of hormone receptors and *HER2* overexpression (Ristimäki *et al.,* 2002; Subbaramaiah *et al.,* 2002; Denkert *et al*., 2003; Shim *et al*., 2003; Wulfing *et al.,* 2003; Boland *et al.,* 2004; Tan *et al*., 2004; Perrone *et al*., 2005; Takeshita *et al*., 2005; Barnes *et al*., 2006).

Table 19. Risk and protective factors for breast cancer

STRENGTH OF ASSOCIATION	RISK FACTORS	MAGNITUDE OF EFFECT
HIGH	Family history in a first-degree relative	++
	Height	++
	Benign breast disease	++
	Breast density	++
	Age at first birth (≥30 vs ≤20)	++
	Menopause ≥54 vs ≤45	++
	High endogenous estrogen levels	++
	Hormone replacement therapy	+
	Exposure to ionizing radiation	++
	Menarche 12≤ vs ≥14	+
	Daily alcohol intake	+
	High BMI after menopause	+
	High BMI before menopause	-
	Tamoxifen	-
MODERATE	High endogenous androgen levels	++
	Current contraceptive use	+
	Physical activity	-
	Prolonged breastfeeding	-
	Folate intake	-
	Carotenoid intake	-
WEAK/ NON-EXISTENT	Fat intake at adult age	·
	Abortion/miscarriage	·
	Tobacco	·
	Past contraceptive use	·
	Exposure to electromagnetic fields	·
INCONCLUSIVE	High endogenous prolactin levels	++
	High IGF levels	++
	High endogenous progesterone levels	+
	High endogenous vitamin D levels	-
	Obesity during childhood	-
	In-utero exposures	+
	NSAIDs	-
	Exposure to organochlorates	·
	Type 2 diabetes mellitus	+
	Thyroid disease	+

From Hankinson, 2008

Similarly, a high rate of *COX-2* overexpression has been found in DCIS, a premalign breast lesion. This fact might make COX/PG pathway a potentially useful target to prevent DCIS progression towards invasive disease (Boland *et al.,* 2004; Half *et al.,* 2002; Soslow *et al.,* 2000; Watanabe *et al.,* 2003; Shim *et al.,* 2003; Tan *et al.,* 2004). *COX-2* expression has also been found in focal areas of healthy breast tissue, in association with silencing of *CDKN2A* ($p16^{INK4a}$), which would mean that COX-2 overexpression is a very early event in breast tumorogenesis (Crawford *et al.,* 2004).

PGE_2, the main product of *COX-2*, is also found in higher concentrations in neoplastic breast tissue (Bennett *et al.,* 1983) (Figure 4). Furthermore, *COX-2* activation in breast tumors seems to be exclusively confined to epithelial cells (Hamid *et al.,* 1999; Howe *et al.,* 2001; Howe *et al.,* 2002; Nakatsugi *et al.,* 2000; Robertson *et al.,* 1998), in contrast with data from colorectal cancer models, in which *COX-2* overexpression has been identified in the stromal component of intestinal adenomas (Oshima *et al.,* 1996). The association between high PGE_2 concentrations and breast tumorogenesis seems to relate to an increase in aromatase activity, which in turn leads to an increase of estrogen synthesis in the epithelium and stromal cells (Brueggemeier *et al.,* 2005). High levels of prostaglandins, derived from the activation of the COX/PG pathway, contribute to carcinogenesis in various ways (increase of mitogenesis, mutagenesis, angiogenesis, metastasis formation, inhibition of apoptosis and immunosupresion).

Conversely, both genetic and pharmacological blockages of COX-2 in the experimental setting have demonstrated that *COX-2* inhibition suppresses breast cancer and that *COX-2* overexpression leads to tumor formation. As expected, the use of COX-2 inhibitors *in vitro* and in animal models has also shown a cancer suppressor activity. Interestingly, however, these drugs seem to exert an anti-cancer activity through COX-independent mechanisms, as well (McCormick and Wilson, 1986).

The fact that *COX-2* is overexpressed in murine models of breast tumors turn these animals into a highly useful experimental tool to assess the role of COX enzymes. During the last three decades, numerous studies have demonstrated breast cancer suppression by inhibiting *COX* activity with traditional NSAIDs and COX-2 inhibitors in the experimental setting, suggesting their chemopreventive effect against breast cancer development. On the other hand, genetic ablation of *COX-2* decreases tumor formation (Howe *et al.,* 2001; Howe, 2005).

Surprisingly, transgenic *COX-2* overexpression is enough to induce breast tumors in multiparous animals, providing direct evidence of *COX-2* oncogenic potential *in vitro* (Liu *et al.,* 2001). Therefore, approaches based on animal models have played a main role in terms of establishing *COX-2* contribution to breast cancer.

Regarding hormone-receptor status, there is additional evidence on the efficacy of *COX-2*-inhibitors in models with estrogen-negative receptors (Boland *et al.,* 2004; Denkert *et al.,* 2003; Wulfing *et al.,* 2003, Ristimäki *et al.,* 2002). Some studies including *HER2* transgenic mice, have found a significant delay in ER-negative tumor formation upon celecoxib administration (Howe *et al.,* 2002; Lanza-Jacobi *et al.,* 2003). This information suggests that blockage of COX/PG pathway could be useful not only for both *HER2/neu*-overexpressing tumors, but also for ER-negative tumors.

In summary, animal models of carcinogenesis provide compelling evidence that NSAIDs inhibit growth and development of breast tumors, which in turn supports the validity of the COX/PG pathway as anticancer target. While preclinical investigations provide consistent evidence that both selective and nonselective NSAIDs inhibit chemically induced carcinogenesis of mammary epithelial tumors, the strongest antineoplastic effects are clearly the result of intervention by administration of COX-2 blocking agents. Although cardiovascular toxicity attributed to COX-2-inhibitors has partially decreased their usefulness in cancer prevention, the analysis of PGE_2–related pathways makes it possible to further identify new pharmacological targets for cancer treatment and prevention.

Several possible targets along the eicosanoid metabolic pathway have been identified. In this context, it is important to remark the abundant evidence supporting PGE_2 as the most tumorogenic prostanoid. Thus, it seems reasonable to suggest that selective blockage of PGE_2 synthase or receptors could be as useful against neoplasia in the same way it is useful against pain and inflammation. The focus has been recently set on microsomal prostaglandin E synthase (mPGES-1), which appears activated in numerous human cancers, including breast cancer (Yoshimatsu *et al.,* 2001; Mehrotra *et al.,* 2006). Blocking mPGES-1 does not increase thrombogenesis or blood pressure (Cheng *et al.,* 2006), consistently with the hypothesis that prostacyclin suppression is the key component to COX-2-inhibitor-mediated cardiac toxicity. Therefore, mPGES-1 could represent an alternate target within the COX-2 pathway to fight inflammation and cancer.

The individual role of PGE_2 receptors (EP_1 to EP_4) in cancer is also under research. Their expression has been identified in murine mammary tumors (Howe *et al.,* 2002; Chang *et al.,* 2004). Genetic and pharmacological blockage of these receptors has been used in multiple animal models to assess the contribution of each receptor to tumorogenesis, although no significant differences among the receptors has been found in this respect (Fulton *et al.,* 2006). EP_1, EP_2, and EP_4 seem to present a protumorogenic effect, but there is no evidence on any of them being optimal for anticancer applications.

Expansion of PGE_2 inactivation could represent an alternate mechanism to tackle COX-related neoplasia. PGE_2 is metabolized into relatively inactive forms (15-keto-PGs y 15-keto-lipoxins) under the effect of 15-NAD hidroxyprostaglandin dehydrogenase, more commonly named 15-hidroxyprostaglandin-dehydrogenase (15-PGDH) (Figure 1). Surprisingly, low levels of 15-PGDH have been observed in multiple tumors, such as non-small-cell lung cancer, colorectal cancer and breast cancer, with abundant evidence on 15-PGDH-action as tumor blocker (Blacklund *et al.,* 2005; Ding *et al.,* 2005; Myung *et al.,* 2006; Wolf *et al.,* 2006; Yan *et al.,* 2004; Mann *et al.,* 2006). These findings suggest the interesting possibility that the PGE_2 pathway could be blocked by reverting the epigenetic inactivation of the *15-PGD*-locus, which leads to a new approach to PGE_2-mediated neoplas

It is important to highlight that all the mechanisms above mentioned, and the ones previously explained, probably work in combination with each other and/or in a synergistic fashion with other cancerogenesis pathways. For example, some carcinogens present in tobacco smoke are mutagenic in mammary tissues (Salaspuro, 2009) and acetaldehyde, the primary metabolite of alcohol metabolism has demonstrated a powerful mutagenic impact in all tissues studied (McGettigan and Henry, 2011).

However, the promising experimental findings do not have a strong epidemiological correlate. A considerable amount of studies have been published during the last 35 years and their results have been irregular, including a high proportion of non-significant results, although they globally support a slightly protective effect of NSAIDs against breast cancer. It is important to highlight the practical absence of randomized controlled trials and the fact that observational studies are based on self-reported use of NSAIDs. Among observational studies, case-control studies tend to report stronger effects than cohort studies. Although well-organized case-control studies would be as accurate as cohort ones, it seems on empirical

basis that case-control studies are exposed to more frequent biases such as recall bias or selection bias. Therefore, the effect size of NSAID on breast cancer incidence would be lower than reported here. This fact is especially relevant for those effects only reported in case-control studies, as occurs with COX-2 selective inhibitors or ibuprofen. Cohort sudies, however, on the other hand, rarely update the information provided by the participants at baseline -only 13 studies provided updated information on NSAID use (Egan *et al.,* 1996; Sharpe *et al.*, 2000; Friis *et al.,* 2002; Friis *et al.,* 2003; Sørensen *et al., 2003;* García-Rodríguez and González-Pérez, 2004; Jacobs *et al.,* 2005; Jacobs *et al.,* 2007; Bardia *et al.,* 2007; Ready *et al.,* 2008; Friis *et al.,* 2008; Eliassen *et al.,* 2009; Zhang *et al.,* 2012), and this information was obtained, in many cases, through prescription records, which are not tantamount to real use (Sharpe *et al.*, 2000; Friis *et al.,* 2002; Friis *et al.,* 2003; Sørensen *et al., 2003;* García-Rodríguez and González-Pérez, 2004; Friis *et al.,* 2008). This means that NSAID consumption refers to that reported many years before breast cancer occurrence. If the protective effect of NSAID is only observed among current users, many cohort studies may suffer from an important degree of misclassification when assessing the relevant exposure (Tables 20 and 21).

Table 20. Results and characteristics from case-control studies included in this meta-analysis

Source	Country	Type of NSAID	No. of case/ control subjects	Type of control	Measurement	Confounding variables	OR (95% CI, any intake)	OR (95% CI, highest intake)
Harris *et al.*, 1995	USA	Any	744/767	Hospital *(Cancer/ non-cancer)*	*Duration: 1-4, ≥5**y*** *Frequency: ≥3/week*	*Age, parity, family history, menopausal status, **BMI**, **OA**, chronic headache, cardiovascular disease*	1.12 (0.8-1.6)	0.58 (0.4-0.8)
Harris *et al.*, 1995	USA	Any ***OTC** (aspirin, ibuprofen)* ***Prescription***	303/906	Population	*Duration (≤1y, ≤5y, ≥5y) Frequency (3-6, ≥7/week)*	*Age, parity, menopausal status, family history*	0.65 (0.5-0.9)	0.60 (0.4-0.9)
Rosenberg, 1995	USA	Any	4485/8391	Hospital	≥4 days/week for ≥3 months, initiation ≥6 months earlier	*Age, gender, interview year, geographic area, race, religion, alcohol, coffee, cholecystectomy, family history of large bowel cancer, education, no. of hospitalizations*	0.8 (0.6-1.0)	-
Harris *et al.*, 1996	USA	Any *Aspirin Ibuprofen*	511/15	Population	*Frequency: 3-6, ≥7 pills/week Duration: ≥1, <5, ≥5 y*	*Age, race, marital status, education*	0.66 (0.52-0.83) *0.69 (0.46-0.99)* *0.66 (0.52-0.83)*	0.60 (0.40-0.91) - -
Neugut *et al.*, 1998	USA	Aspirin	252/322	Hospital	*[not provided]*	*Age, gender, race, smoke, prior **CHD**, diabetes, menopausal status*	0.80 (0.35-1.80)	-
Coogan *et al.*, 1999	USA	Any	6558/2925	Hospital	*Duration: <1, 1-2, 2-5, 5-10, 10-20, ≥20y Regularity*	*Age, center, year of interview, education, benign breast disease, doctor visits before hospitalization, **HRT**, contraceptives, age at menarche, age at menopause, age at first birth, parity, race, alcohol, religion, family history, self examinations, **BMI***	0.70 (0.60-0.90)	0.6 (0.3-1.0)
Langman *et al.*, 2000	UK	Any	3105/9272	Hospital	*No. of prescriptions: 0, 1, 2-6, ≥7 Duration: 13-24, 25-36 months*	*Age, smoke*	1.01 (0.93-1.10)	1.12 (0.90-1.40)
Cottercchio *et al.*, 2001	Canada	Any *Aspirin*	3133/3062	Population	*Duration: ≤1, 2-8, ≥9y Time since last use: ≤1, 2-6, ≥7y*	*Age, family history, benign breast disease, age at menarche, parity, age at*	0.76 (0.66-0.88) *0.73 (0.61-0.87)*	0.68 (0.54-0.86) -

		Non-aspirin			*Age at first use: ≤43, 44-49, ≥50 y*	*menopause, **HRT**, education, marital status*	*0.79 (0.66-0.96)*	-
Meier *et al.,* 2002	UK	Any Acetaminophen	3706/14155	Population	*No. of prescriptions: 1-9, 10-19, 20-29, ≥30*	*Age, **BMI**, smoke*	1.00 (0.9-1.1) 1.00 (0.9-1.1)	1.0 (0.8-1.1) 0.8 (0.7-1.0)
Moorman *et al.,* 2003	USA	Any	930/754	Population	*Regularity: any, occasional, regular use* *Duration: <3, ≥3y*	*Age, race, age at menarche, age at first birth, breastfeeding, menopausal status, family history, contraceptives, **HRT**, education, **BMI**, waist:hip ratio, alcohol, smoke*	0.4 (0.3-0.6)	0.3 (0.2-0.5)
Terry *et al.,* 2004	USA	Aspirin Ibuprofen Acetaminophen	1442/1420 1443/1420 1434/1417	Population	*Duration: <5, ≥5y* *Frequency: <7, ≥7 times/week* *Regularity: regular, nonregular use* *Time: current, former use*	*Age at diagnosis, migraine, **BMI**, use of other medication*	0.80 (0.66-0.97) 0.91 (0.72-1.16) 1.02 (0.80-1.31)	0.77 (0.57-1.04) 1.09 (0.70-1.70) 0.91 (0.58-1.41)
Rahme *et al.,* 2005	Canada	Cox-2- inhibitors Non-aspirin Aspirin Acetaminophen	1090/44990	Population	*Duration: ≥90days* *Dose (aspirin only): ≤100 mg/day, > 100 mg day*	*Age, recent mammogram, recent breast procedure, breast disease, **HRT**, recent visit to a gynecologist*	0.81 (0.68-0.97) 0.65 (0.43-0.99) 0.75 (0.64-0.89) 0.91 (0.71-1.16)	- - - -
Swede *et al.,* 2005	USA	Aspirin	1478/3383	Hospital	*Regularity: occasional/regular* *Frequency: 1, 2-6, ≥7 tablets/week* *Duration: 1-9, ≥10 y* *Tablet-years: ≤10, ≥11*	*Age, age at menarche, parity, age at first birth, menopausal status, **BMI**, education, family history, benign breast disease*	0.83 (0.75-0.93)	0.85 (0.75-0.96)
Zhang *et al.,* 2005	USA	Any *Aspirin* *Ibuprofen*	7006/3622	Hospital	*Regularity: nonregular/regular* *Time: <1, ≥1 y before admission (continued/discontinued)* *Duration: <1, 1-2, 2-5, 5-10, 10-20, ≥20 y*	*Age, year of interview, study center, race, education, benign breast disease recent physician visits, **HRT** contraceptives, age at menarche age a menopause, age at first birth, parity, alcohol, family history, self-examination, **BMI**, **HR** status*	1.01 (0.90-1.13)	0.62 (0.28-1.35) *0.59 (0.25-1.36)* *0.78 (0.29-2.08)*
Harris *et al.,* 2006	USA	Cox-2-inhibitors Aspirin Baby aspirin Ibuprofen/naproxen Acetaminophen	323/649	Hospital	*Frequency: 2-3, >3 weekly*	*Age, race, education, parity, family history, BMI, menopausal status, smoke, alcohol*	0.29 (0.14-0.59) 0.49 (0.26-0.94) 0.82 (0.40-1.40) 0.37 (0.18-0.72) 1.02 (0.39-2.20)	- 0.39 (0.22-0.72) - - -
Davis and Mirick, 2007	USA	Any	600/647	Population	*Duration: <5, ≥5y*	*Age, parity, age at first pregnancy,*	1.1 (0.8-1.4)	1.0 (0.7-1.5)

					Time: ≤2, >2 y before diagnosis	*family history, early double oophorectomy, contraceptives, menopausal status, ever **g-i** series, smoke, alcohol, **HRT***		
Kirsh *et al.,* 2007	Canada	Any *Aspirin* *Non-aspirin*	3125/ 3062	Population	*Duration: ≤1, 2-6, ≥7 y* *Time of last use: ≤1, 2-6, ≥7y*	*Age, family history, migraine, arthritis, **HRT**, **BMI**, smoke, menopausal status, exercise, alcohol, education, **HR** status*	0.76 (0.66-0.88)	-
Slattery *et al.,* 2007	USA	Aspirin	2325/ 2525	Population	*Regular use (≤3 weekly for ≤1 month)*	*Age, center, genetic admixture, parity, **BMI**, exercise, menopausal status, education*	0.94 (0.82-1.07)	-
Brasky *et al.,* 2010	USA	Aspirin Ibuprofen Acetaminophen	1170/ 2115	Population	*Frequency: <14, ≥14 days/month, <2, ≥2 pills/day* *Average monthly frequency during decades (aspirin only): <2, ≥2 pills/month*	*Age, education, age at menarche, age at menopause, age at first pregnancy, parity, **BMI**, race, menopausal status, **HRT**, benign breast disease, family history, hypertension, **CVD**, arthritis*	0.80 (0.68-0.94) 1.15 (0.97-1.36) 0.97 (0.83-1.15)	0.68 (0.46-1.00) 1.12 (0.94-1.34) 1.01 (0.85-1.20)
Cronin-Fenton *et al.,* 2010	Denmark	Any *Cox-2-inhibitors* *Non-selective NSAIDs* *Aspirin*	8195/ 81950	Population	*Time: recent, former use* *Duration: <10, 10-15, ≥15 y* *Prescription use/duration: <25%, 25-50%, >50%*	*Age, **HRT**, **RA**, migraine*	1.04 (0.99-1.10) *1.08 (0.99-1.18)* *1.04 (0.98-1.10)* *0.96 (0.87-1.06)*	1.01 (0.52-1.97) - - -
Ashok *et al.,* 2011	USA	Non-selective Celecoxib Rofecoxib Valdecoxib Acetaminophen	18368/ 73472	Population	*Duration: any, <6, 7-12, 12-24, >24 months* *Duration of continuous dose: <6, 7-12, ≥12 months*	*Age, contraceptives*	0.85 (0.82-0.88) 0.86 (0.81-0.91) 0.68 (0.62-0.74) 0.81 (0.71-0.9) 0.95 (0.85-1.06)	0.78 (0.69-0.89) 0.84 (0.73-0.97) 0.59 (0.46-0.76) 0.94 (0.52-1.68) 1.09 (0.61-1.92)
Vinogradova *et al.,* 2011	UK	Cox-2-inhibitors	15666/ 88125	Population (nested)	*Duration: <90, 90 days-12 months, 13-24 months, ≥25 months*	*Gender, age, deprivation, **BMI**, smoke, comorbidities, medications*	1.24 (1.08-1.42)	1.19 (0.98-1.44)
Ou *et al.,* 2013	Taiwan	Any	11/36	Hospital (nested)	*≥28 **cDDDs***	*Age, gender, hemodialysis, economic status, urbanization, comorbidities, concomitant medications*	0.41 (0.19-0.89)	-

***BMI**: body mass index; **cDDDs**: cumulative defined daily dose; **CHD**: congestive heart disease; **CI**: confidence interval; **CVD**: cardiovascular disease; **G-i**: gastrointestinal; **HR**: hormone receptor; **HRT**: hormone replacement therapy; **OA**: osteoarthritis; **OCT**: over-the-counter; **OR**: odds ratio; **prescription**: naproxen, indomethacin, piroxicam; **pys**: person-years; **RA**: rheumatoid arthritis; **RCT**: randomized controlled trial; **y**: year*

Table 21. Results and characteristics from cohort studies included in this meta-analysis

Source	Country	Type of NSAID	No. of cases/ cohort size	Follow-up period	Measurement	Confounding variables	RR (95% CI, any intake)	RR (95% CI, highest intake)
Paganini-Hill *et al.*, 1989	USA	Aspirin	214/8818	*>42000* ***pys***	*Frequency: none, <daily, daily*	*Gender, age*	0.96	-
Schreinemachers & Everson, 1994	USA	Aspirin	174/11411	*85002 pys*	*Date of last aspirin intake (last 30 days)*	*Gender, age, race, education, smoking, alcohol, poverty,* ***BMI****, arthritis*	0.72 (0.52-1.00)	-
Egan *et al.*, 1996	USA	Aspirin	2414/89528	*1020774 pys*	*Years of regular use (≥2/week)*	*Age*	1.01 (0.80-1.27)	1.12 (0.76-1.66)
Harris *et al.*, 1999	USA	Any Aspirin Acetaminophen Ibuprofen	393/32505 76/32505 36/32505 37/32505	*4.7* ***y*** *152496 pys*	*Frequency:* *<1, 1-3, ≥4/week*	*Age, education, parity, menopausal status, family history*	0.64 (0.50-0.82) 0.57 (0.40-0.81) 0.84 (0.55-1.18) 0.53 (0.33-0.84)	0.57 (0.44-0.74) 0.64 (0.45-0.90) 0.84 (0.47-1.50) 0.49 (0.30-0.80)
Sharpe *et al.*, 2000	USA	Any	5882/25317	*3.7 y for cases* *3.8 y for controls*	*Time of last exposure:* *1–6 m, 7–12 m, 2–5 y,* *6–10 y, 11–15 y* *Dose: 0>$\Sigma p_i \leq 0.1$; 0.1<$\Sigma p_i \leq 0.3$;* *$\Sigma p_i \geq 0.3$* *(p_i=dispensed/ recommended daily dose)*	*Oral contraceptives, corticosteroids, estrogens,* ***OTC*** *ibuprofen/aspirin, tobacco, alcohol, family history, benign breast biopsies, education, age at menarche, age at first birth, duration of lactation, height,* ***BMI*** *before menopause,* ***BMI*** *after menopause, age at menopause*	0.95 (0.91-0.99)	0.91 (0.75-1.09)
Friis *et al.*, 2002	Denmark	Acetaminophen	227/39946	*3.9 y* *38888 pys*	*No. prescriptions: 1, 2-4, 5-9, ≥10*	*Gender, age at entry*	1.0 (0.9-1.2)	-
Johnson *et al.*, 2002	USA	Any	938/27616	*190000 pys*	*Frequency* *<1, 1, 2-5, ≥6/week*	*Age,* ***BMI****, waist:hip ratio, benign breast disease, family history relative, current estrogen use, multivitamin use*	0.80 (0.67-0.95)	1.01 (0.83-1.25)
Friis *et al.*, 2003	Denmark	Aspirin	149/29470	*4.1 years*	*No. of prescriptions: 1, 2-4, 5-9, ≥10* *Duration <1, 1-4, 5-9 years*	*Gender, age at entry*	0.9 (0.8-1.1)	-
Harris *et al.*, 2003	USA	Any *Aspirin* *Acetaminophen* *Ibuprofen* *Prescription NSAIDs**	1392/80741	*43 months* *194884 pys*	*Duration <1, 1-4, 5-9, ≥10 y*	*Age, ethnicity, education,* ***BMI****,* ***HRT****, family history, parity before 30, weekly exercise*	0.93 (0.78-1.10) *0.90 (0.72-1.13)* *1.02 (0.75-1.37)* *0.83 (0.63-1.10)* *1.14 (0.79-1.62)*	0.81 (0.68-0.97) *0.81 (0.66-0.99)* *0.96 (0.76-1.20)* *0.82 (0.60-1.12)* *0.64 (0.36-1.17)*
Sorensen *et al.*, 2003	Denmark	Any	696/172057	*5.4 y* *751182 pys*	*No. of prescriptions: 1, 2-4, 5-9, ≥10*	*Gender*	1.1 (1.0-1.2)	1.1 (0.9-1.3)

Ratnasinghe *et al.*, 2004	USA	Aspirin	131/12834	-	*Time since last use* *Intake in the last 6 months (1/week or >1/week)*	*Age, **BMI**, gender, race, poverty, education, smoking*	0.82 (0.49-1.36)	-
García-Rodríguez and González-Pérez, 2004	UK, Spain	Aspirin Non-aspirin NSAIDs Acetaminophen	3708/734899	100000 pys	*Time of use: none, current (<1, 1-1.9, 2-3.9, ≥4y), past Dose/preparation Indication*	*Age, alcohol use, smoking, **BMI, HRT**, previous breast abnormalities*	0.84 (0.69-1.02) 0.98 (0.88-1.09) 0.92 (0.83-1.03)	0.87 (0.53-1.41) 1.05 (0.80-1.38) 0.76 (0.60-0.97)
Cook *et al.*, 2005; Zhang *et al.*, 2008 (**RCT**)	UK	Aspirin	1230/39884	*10.1 y* *100000 pys*	*100 mg/48h, 138 months*	*Age, **BMI**, smoking, alcohol, exercise, menopausal status/**HRT**, family history, vitamin E, ß-carotene*	0.98 (0.87-1.09)	-
Jacobs *et al.*, 2005	USA	Any *Aspirin* *Iburprofen* *Other*	3008/77413	*549044 pys*	*Pills/month: 1-14, 15-29, 30-59, ≥60* *Time/duration: past use, current regular use (<5, ≥5 y)*	*Age, menopausal status, race, **BMI**, weight gain/loss since age 18, **HRT**, most recent mammogram*	1.16 (1.02-1.31) *1.08 (0.94-1.23)* *1.02 (0.79-1.33)* *1.17 (0.89-1.53)*	1.05 (0.88-1.26) *0.88 (0.69-1.12)* *1.29 (0.92-1.82)* *0.90 (0.58-1.40)*
Marshall *et al.*, 2005	USA	Any Acetaminophen Ibuprofen Aspirin	2391/114640	*6 y*	*Frequency: 1-6 days/week; daily* *Duration: <5, ≥5y*	*Race, family history, **BMI**, smoking, alcohol, exercise, socioeconomic status, number of births, parous before 30, menopausal status, **HRT**, breast biopsy history, mammogram in the previous 2 years*	- - - -	1.11 (0.96-1.30) 0.96 (0.63-1.47) 1.51 (1.17-1.95) 0.96 (0.79-1.18)
Gallichio *et al.*, 2007	USA	Any Acetaminophen	418/15651	*12 y*	*Time of use (previous 48h)* *Frequency (once a week for ≥1 year): <1/≥1 daily* *Dose* *Duration: <5, ≥5y*	Age, education, family history, ever pregnant, age at first birth, age at menarche, height, weight, **BMI** before and after menopause, smoking, alcohol	0.89 (0.72-1.09) 0.94 (0.71-1.25)	- -
Gill *et al.*, 2007	USA	Any Acetaminophen	3493/98920 278/98920	*9 y*	*Duration <2, 2-5, 6-10, ≥11 y* *Time: current, past use*	*Age, ethnicity, family history, recent mammogram, education, alcohol, age at menarche, age at first birth, parity, age and type of menopause, **HRT**, **BMI**, **HR** status, past pain medication use, risk of breast cancer*	0.88 (0.75-1.04) 1.14 (0.91-1.42)	0.99 (0.82-1.18) 1.05 (0.83-1.33)
Jacobs *et al.*, 2007	USA	Aspirin	3121/76303	*100000 pys*	*Dose: "baby" vs adult* *Frequency: days/month during last year; pills/day* *Duration: years of use*	*Age, race, education, smoking, exercise, non-aspirin NSAIDs, heart attack, diabetes, hypertension, **BMI***	1.02 (0.88-1.19)	0.83 (0.63-1.10)
Bardia *et al.*, 2007	USA	Aspirin Non-aspirin NSAIDs Combined use	3487/22507	*10 y* *226798 pys*	*Frequency: ≤1, 2-5, ≥6 times/week*	*Age, **BMI**, waist:hip ratio, diet, education, alcohol, exercise, estrogen use, **RA**, **OA**, smoke*	0.84 (0.77-0.90) 0.96 (0.89-1.04) 0.81 (0.72-0.90)	0.81 (0.73-0.90) 0.94 (0.83-1.06) -
Friis *et al.*, 2008	Denmark	Any	847/28695	*7.5 y*	*No. of prescriptions: 0, 1, 2-4, 5-9, 10-*	*Age, education, **BMI**, menopausal status, parity, age at*	1.51 (1.04–2.20)	1.32 (1.13–1.54)

		Aspirin *Non-aspirin* *Acetaminophen*		*2149557 pys*	*19, ≥20*	*first birth, exercise, benign breast disease, alcohol, **HRT**, **HR** status*	*1.40 (1.13–1.75)* *1.18 (0.98–1.43)* *1.02 (0.77–1.36)*	- - -
Ready *et al.,* 2008	USA	Any (except low-dose aspirin) *Low-dose aspirin* *Regular aspirin* *Non-aspirin* *Ibuprofen* *Naproxen*	482/35323	*4 y*	*Duration: 1-3, 4-8, 9-10 y* *Frequency: 1-3, ≥4 days/week*	*Age, race, **BMI**, inflammatory conditions, **HRT**, tumor size, **HR** status, education, age at menarche, age at first birth, age at menopause, surgical menopause, family history, recent mammogram, breast biopsies, multi-vitamin use, exercise, alcohol, diet*	0.98 (0.67-1.44) *0.99 (0.80-1.23)* *0.96 (0.76-1.22)* *0.96 (0.78-1.18)* *1.05 (0.85-1.31)* *0.88 (0.64-1.21)*	1.26 (0.96-1.65) *0.65 (0.43-0.97)* *1.43 (1.02-2.00)* *1.28 (0.91-1.80)* *1.23 (0.79-1.92)* *1.72 (0.93-3.16)*
Gierarch *et al.,* 2008	USA	Any *Aspirin* *Non-aspirin* *Combination*	4501/126124	*836863 pys*	*Time of use: during last year* *Frequency: <1 week, 1-6 week, ≥1 daily*	*Age, race, age at first birth, **HRT**, breast biopsies, alcohol, hypertension, family history, **HR** status*	0.97 (0.88-1.07) 0.95 (0.87-1.04) 1.01 (0.92-1.12) 0.95 (0.87-1.04)	- - - -
Siemes *et al.,* 2008	Netherlands	Any *Non-aspirin* *Aspirin* *Non-selective* *COX-1 selective* *COX-2 selective*	175/7621	*9.7 y*	*Any use, 1-365, >365 days*	*Age, gender, **BMI**, smoke, exercise, **RA**, **OA**, C-protein, diet, age of menarche, age of menopause, **HRT**, parity*	1.19 (0.81-1.73) *1.18 (0.81-1.72)* *0.94 (0.52-1.70)* *1.18 (0.80-1.72)* *1.25 (0.73-2.15)* *0.90 (0.29-2.83)*	1.27 (0.80-2.00) *1.40 (0.79-2.50)* *1.16 (0.67-2.02)* *1.37 (0.72-2.62)* *0.99 (0.28-3.49)* *1.93 (0.17-21.61)*
Eliassen *et al.,* 2009	USA	Aspirin Non-aspirin NSAIDs Acetaminophen	1345/112292	*1241823 pys*	*Time of use: past, current* *Duration: <5, ≥5y* *Frequency: 1, 2-3, 4-5, ≥6/week*	*Age, age at menarche, height, **BMI** at age 18, weight change since age 18, contraceptives, parity, age at first birth, alcohol, benign breast disease, family history, **HR** status*	1.07 (0.89-1.29) 1.16 (1.01-1.34) 0.99 (0.84-1.16)	1.03 (0.74-1.42) 0.86 (0.60-1.24) 1.06 (0.64-1.76)
Bardia *et al.,* 2011	USA	Aspirin Non-aspirin NSAIDs Combined use	1581/26580	*307178 pys*	Frequency: *≤1, 2-5, ≥6/week*	*Age, education, family history, age at menarche, age at menopause, parity, age at first birth, contraceptives, **HRT**, **BMI**, weight at age 12, **OA**, **RA**, alcohol, smoke, exercise, **HR** status*	0.80 (0.71-0.90) 0.95 (0.85-1.07) 0.77 (0.65-0.91)	0.71 (0.60-0.83) 1.00 (0.84-1.19) -
Zhang *et al.,* 2012	USA	Aspirin Non-aspirin NSAIDs Acetaminophen	4734/84602	*28 y*	*Time of use: current, past* *Frequency: tablets/week, days/week* *Duration ≤5, 6-10, 11-20, ≥20 y*	*Age, age at menarche, age at first birth, nulliparity, height, **BMI** at age 18, weight change since age 18, exercise, family history, benign breast disease, alcohol, **HRT**, smoke*	0.91 (0.81-1.01) 0.97 (0.90-1.04) 0.89 (0.83-0.96)	-

***BMI**: body mass index; **cDDDs**: cumulative defined daily dose; **CHD**: congestive heart disease; **CI**: confidence interval; **CVD**: cardiovascular disease; **G-i**: gastrointestinal; **HR**: hormone receptor; **HRT**: hormone replacement therapy; **OA**: osteoarthritis; **OCT**: over-the-counter; **OR**: odds ratio; **prescription**: naproxen, indomethacin, piroxicam; **pys**: person-years; **RA**: rheumatoid arthritis; **RCT**: randomized controlled trial; **y**: year.*

Moreover, many of these drugs can be obtained over-the-counter, so their consumption is even more difficult to record; the few exceptions are studies based on prescriptions, which constitute a safer strategy to assess their sale but they do not necessarily assess NSAID consumption. Another possible explanation for the disparities in results may lie on the fact that some antiinflammatory drugs inhibit COX-2 more intensely than others, which leads to different risk reductions. Finally, the hypothesis that different COX-2 genotypes, different hormone receptor patterns, and the presence of inflammatory disease may modify the effect of NSAIDs in each individual risk might also account for some of the aforementioned heterogeneity.

This meta-analysis intended to answer at least some of these questions. Globally, the results confirm that consumption of NSAIDs reduces the risk of invasive breast cancer by about 20%. A similar effect was found for consumption of specific antiinflammatory or analgesic drugs such as aspirin, acetaminophen, COX-2 inhibitors and, to a lesser extent, ibuprofen. Although similar results had been reported in previous meta-analyses, our study updates this information including recent studies.

The most innovative results of this meta-analysis are the protective effect of COX-2 inhibitors on breast cancer (OR 0.90) and the protective effect of aspirin in preventing specifically ER+ and PR+ breast tumors (OR 0.73 in both cases). To our best knowledge, such results have not been reported previously in any meta-analysis.

Data concerning specific COX-2 inhibitors are still scarce (Rahme *et al.*, 2005; Harris *et al.,* 2006; Cronin-Fenton *et al.*, 2010; Ashok *et al.*, 2011; Vinogradova *et al.,* 2011), mainly due to discontinuation of their use after observing they were linked to an increase of thromboembolic cardiovascular risk. Nevertheless, their effect on reducing breast cancer risk seems stronger than that of traditional NSAIDs and recent reviews have reported their use to be safe if dosage is within a certain range (Coogan *et al.*, 1999). Further studies are required to confirm the effect of COX-2 inhibitors in reduction of breast cancer risk, specifically regarding the differential effect of these drugs in HR positive and HR negative breast cancer.

Similarly, few studies have been published in which different molecular types of breast cancer and hormonal receptor status are considered (Terry *et al.*, 2004; Zhang *et al.*, 2005; Kirsh *et al.*, 2007; Brasky *et al.*, 2011; Marshall *et al.*, 2005; Gallicchio *et al.*, 2007; Friis *et al.*, 2008; Eliassen *et al.*, 2009; Bardia *et al.*, 2011; Zhang *et al.,* 2012; Zhang *et al.*, 2008). They had

only been partially included in previous meta-analyses either because they were unpublished (Zhang *et al.*, 2005; Kirsh *et al.*, 2007; Brasky *et al*., 2011,; Gallicchio *et al.,* 2007; Gill *et al.,* 2007; Friis *et al.*, 2008; Eliassen *et al.*, 200; Bardia *et al.,* 2011; Zhang *et al.*, 2012; Zhang *et al.*, 2008) or because data were insufficient for a meta-analysis (Jonsson *et al.*, 2013; Eliassen *et al.,* 2009). While two recent meta-analyses published in 2012 (Luo *et al.,* 2012; Tolentino *et al.*, 2012) include some of these studies (Terry *et al.*, 2004; Zhang *et al.,* 2005; Kirsh *et al.*, 2007; Brasky *et al.*, 2010; Marshall *et al.*, 2005; Gill *et al.,* 2007; Friis *et al.*, 2008; Gierarch *et al.*, 2008; Eliassen *et al.,* 2009; Bardia *et al.*, 2011) they restricted the analysis to the effect of aspirin use.

By the time our review was performed, 12 publications were available on the differential effect of NSAIDs on hormone-receptor positive breast cancer, which made it possible to obtain separate results. We observed that NSAID use led to a higher decrease in the risk of ER+ than in breast cancer altogether (i.e.: without specifying the presence or absence of hormonal receptors). Prostaglandin E2 can induce binding of several transcription factors (phosphorylated ATF-2, LRH-1, and C/EBPβ) to aromatase promoters I.3 and II, which induces up-regulating aromatase expression in adipose tissue fibroblasts. Moreover, aromatase is associated to higher exposure to estrogens in breast cancer cells (Zhao *et al.*, 2009). Use of COX-2 inhibitors would down regulate aromatase expression leading to a decrease in breast cancer risk.

LIMITATIONS OF THIS STUDY

Several limitations of our meta-analysis must be taken into account. First of all, we have not studied the effect of different NSAID doses or duration of use because original articles reported this information in very heterogeneous ways; although some meta-analyses have performed a dose-response analysis, we do believe that the lack of standardization in reporting doses or time of exposure makes such analyses unreliable.

Second, several articles reported odds ratios on “any NSAID” without clarifying the composition of that category. In our meta-analysis, we have combined those results, regardless of the possible heterogeneity of such a group. Nevertheless, this heterogeneity should be considered in order to carefully interpret its results. Additionally, NSAID use is not uniformly recorded through the different original articles, including self-reported use, NSAID

prescriptions, or over-the-counter NSAID sales, which leads to an additional source of heterogeneity or bias.

Third, some molecular features are not homogeneously reflected on the studies. For instance, HER2 expression has not been investigated in most publications, which avoids to include it in our meta-analysis in spite of its putative relevance for the NSAIDs protective mechanism. In the same way, some results regarding ER/PR expression refer to the positivity of any of the two receptors without more specification, while other studies consider them separately. The lack of data on hormone receptor status is particularly high in cohort studies -only 7 include this information (Gill *et al.,* 2007; Friis *et al.,* 2008; Ready *et al.,* 2008, Gierarch *et al.,* 2008; Eliassen *et al.,* 2009; Bardia *et al.,* 2011; Zhang *et al.,* 2012)-, which might partially explain the lower risk reductions observed in these studies.

To conclude, further research-worthy hypotheses might ensue from the results of this meta-analysis, such as the possibility that different COX-2 genotypes or inflammatory disease modify NSAID effects, or the specific effect of NSAIDs in each intrinsic molecular subtype of breast cancer.

CONCLUSIONS

1. Use of any non-steroidal antiinflammatory drug and breast cancer risk

The use of any non-steroidal antiinflammatory drug cuts down the risk of breast cancer by 8% in cohort studies and by 18% in case-control studies, regardless of its hormone receptor pattern.

When restricting the analysis to estrogen-receptor positive breast cancers, the pooled odds ratio from case-control studies was lower (0.72). However, the pooled relative risk from the meta-analysis of cohort studies was close to 1, and non-significant.

Data regarding progesterone-receptor positive breast cancers are scarce, and a strong random error remains after pooling them in the meta-analysis. Therefore, although the pooled odds ratio was 0.73, this result was non-significant. The meta-analysis of cohort studies did not revealed any protective effect of non-steroidal antiinflammatory drugs.

2. Use of aspirin and breast cancer risk

Pooling case-control studies, the use of aspirin protected against breast cancer when hormone receptors are not analysed; the odds for breast cancer was 13% lower in women that took aspirins than in women that did not take them. However, this result cannot be reproduced when meta-analysing cohort studies.

Again, after restricting the analysis to estrogen-receptor positive breast cancers, the pooled odds ratio from the case-control studies was significantly lower, showing that aspirin had a protective effect of 27%. Nonetheless, the pooled relative risk from the meta-analysis of cohort studies (0.93) remained close to 1 and non-significant.

The restricted analysis of progesterone-receptor positive breast cancers provides similar results to estrogen-receptor positive breast cancers: the pooled odds ratio was 0.73 in case-control studies and the relative risk was 0.95 when combining cohort studies.

3. Use of ibuprofen and breast cancer risk

The meta-analysis of case-control studies provided an odds ratio of 0.87 for the use of ibuprofen and breast cancer incidence, regardless of its hormone receptor pattern. Only one cohort study was found and its result suggested a harmful effect (RR = 1.09), although it was not significant. Data were insufficient to perform a meta-analysis restricted to estrogen or progesterone-postive receptors breast cancers.

4. Use of specific COX-2 inhibitors and breast cancer risk

The meta-analysis of case-control studies provided an OR of 0.90 for the use of COX-2 inhibitors and breast cancer incidence, regardless of its hormone receptor pattern. Although recent evidence suggests that this effect would be stronger in estrogen-receptor positive breast cancers, there are not enough data yet to perform a meta-analysis.

Altogether, our meta-analysis supports that non-steroidal antiinflammatory drug use has a small protective effect on breast cancer risk, which would be stronger when using COX-2 inhibitors and regarding estrogen-responsive cancer, although the number of studies in this regard is still small. Further research on dose-response effect or duration of use would benefit from standardization in the way such variables are reported in original studies.

REFERENCES

- Abou-Issa HM, Alshafie GA, Harris RE. Chemoprevention of breast cancer by nonsteroidal antiinflammatory drugs and selective COX-2 blockade in animals. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002: 85-98
- Abou-Issa HM, Alshafie GA, Seibert K, Koki AT, Masferrer JL, Harris RE. Dose-response effects of the COX-2 inhibitor, celecoxib, on the chemoprevention of mammary carcinogenesis. Anticancer Research 2001;21:3425–32.
- Adami HO, Signorello LB, Trichopoulos D. Towards an understanding of breast cancer etiology. Semin Cancer Biol 1998;8:255-62.
- Agarwal VR, Bulun SE, Leitch M, Rohrich R, Simpson ER. Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients. J Clin Endocrinol Metab 1996;81:3843–49.
- Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, Platte R, Morrison J, Maranian M, Pooley KA, Luben R, Eccles D, Evans DG, Fletcher O, Johnson N, dos Santos Silva I, Peto J, Stratton MR, Rahman N, Jacobs K, Prentice R, Anderson GL, Rajkovic A, Curb JD, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Diver WR, Bojesen S, Nordestgaard BG, Flyger H, Dörk T, Schürmann P, Hillemanns P, Karstens JH, Bogdanova NV, Antonenkova NN, Zalutsky IV, Bermisheva M, Fedorova S, Khusnutdinova E; SEARCH, Kang D, Yoo KY, Noh DY, Ahn SH, Devilee P, van Asperen CJ, Tollenaar RA, Seynaeve C, Garcia-Closas M, Lissowska J, Brinton L, Peplonska B, Nevanlinna H, Heikkinen T, Aittomäki K, Blomqvist C, Hopper JL, Southey MC, Smith L, Spurdle AB, Schmidt MK, Broeks A, van Hien RR, Cornelissen S, Milne RL, Ribas G, González-Neira A, Benitez J, Schmutzler RK, Burwinkel B, Bartram CR, Meindl A, Brauch H, Justenhoven C, Hamann U; GENICA Consortium, Chang-Claude J, Hein R, Wang-Gohrke S, Lindblom A, Margolin S, Mannermaa A, Kosma VM, Kataja V, Olson JE, Wang X, Fredericksen Z, Giles GG, Severi G, Baglietto L, English DR, Hankinson SE, Cox DG, Kraft P, Vatten LJ, Hveem K, Kumle M, Sigurdson A, Doody M, Bhatti P, Alexander BH, Hooning MJ, van den Ouweland AM, Oldenburg RA, Schutte M, Hall P, Czene K, Liu J, Li Y, Cox A, Elliott G, Brock I, Reed MW, Shen CY, Yu JC, Hsu GC, Chen ST, Anton-Culver H, Ziogas A, Andrulis IL, Knight JA; kConFab; Australian Ovarian Cancer Study Group, Beesley J, Goode EL, Couch F, Chenevix-Trench G, Hoover RN, Ponder BA, Hunter DJ, Pharoah PD, Dunning AM, Chanock SJ, Easton DF. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. Nat Genet 2009;41:585-90.
- Ahn J, Schatzkin A, Lacey JV Jr, *et al*. Adiposity, adult weight change, and postmenopausal breast cancer risk. Arch Intern Med 2007;167:2091-102.
- Akbari MR, Tonin P, Foulkes WD, Ghadirian P, Tischkowitz M, Narod SA. RAD51C germline mutations in breast and ovarian cancer patients. Breast Cancer Res 2010;12:404.
- Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Kato I, Koenig KL, Shore RE. Aspirin and epithelial ovarian cancer. Prev Med 2001;33:682–87.
- Akhurst RJ, Derynck R. TGF-beta signaling in cancer--a double-edged sword. Trends Cell Biol 2001;11:S44-51.
- Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 2012;13:518-27.

- Allen NE, Beral V, Casabonne D, et al. Moderate alcohol intake and cancer incidence in women. J Natl Cancer Inst 2009;101:296.
- Amaral P, Miguel R, Mehdad A, Cruz C, Monteiro Grillo I, Camilo M, Ravasco P. Body fat and poor diet in breast cancer women. Nutr Hosp 2010;25:456-61.
- American Cancer Society. Breast Cancer Facts & Figures 2009-2010, American Cancer Society, Inc, Atlanta.
- Anand S, Penrhyn-Lowe S, Venkitaraman AR. AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. Cancer Cell 2003;3:51-62.
- Anderson GL, Chlebowski RT, Aragaki AK, *et al*. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. Lancet Oncol 2012;13:476.
- Anothaisintawee T, Wiratkapun C, Lerdsitthichai P, Kasamesup V, Wongwaisayawan S, Srinakarin J, Hirunpat S, Woodtichartpreecha P, Boonlikit S, Teerawattananon Y, Thakkinstian A. Risk factors of breast cancer: a systematic review and meta-analysis. Asia Pac J Public Health. 2013;25:368-87.
- Antoniou AC, Sinilnikova OM, Simard J, Léoné M, Dumont M, Neuhausen SL, Struewing JP, Stoppa-Lyonnet D, Barjhoux L, Hughes DJ, Coupier I, Belotti M, Lasset C, Bonadona V, Bignon YJ; Genetic Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers Study (GEMO), Rebbeck TR, Wagner T, Lynch HT, Domchek SM, Nathanson KL, Garber JE, Weitzel J, Narod SA, Tomlinson G, Olopade OI, Godwin A, Isaacs C, Jakubowska A, Lubinski J, Gronwald J, Górski B, Byrski T, Huzarski T, Peock S, Cook M, Baynes C, Murray A, Rogers M, Daly PA, Dorkins H; Epidemiological Study of BRCA1 and BRCA2 Mutation Carriers (EMBRACE), Schmutzler RK, Versmold B, Engel C, Meindl A, Arnold N, Niederacher D, Deissler H; German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC), Spurdle AB, Chen X, Waddell N, Cloonan N; Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab), Kirchhoff T, Offit K, Friedman E, Kaufmann B, Laitman Y, Galore G, Rennert G, Lejbkowicz F, Raskin L, Andrulis IL, Ilyushik E, Ozcelik H, Devilee P, Vreeswijk MP, Greene MH, Prindiville SA, Osorio A, Benitez J, Zikan M, Szabo CI, Kilpivaara O, Nevanlinna H, Hamann U, Durocher F, Arason A, Couch FJ, Easton DF, Chenevix-Trench G; Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). RAD51 135G-->C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet. 2007;81:1186-200.
- Antoniou AC, Wang X, Fredericksen ZS, McGuffog L, Tarrell R, Sinilnikova OM, Healey S, Morrison J, Kartsonaki C, Lesnick T, Ghoussaini M, Barrowdale D; EMBRACE, Peock S, Cook M, Oliver C, Frost D, Eccles D, Evans DG, Eeles R, Izatt L, Chu C, Douglas F, Paterson J, Stoppa-Lyonnet D, Houdayer C, Mazoyer S, Giraud S, Lasset C, Remenieras A, Caron O, Hardouin A, Berthet P; GEMO Study Collaborators, Hogervorst FB, Rookus MA, Jager A, van den Ouweland A, Hoogerbrugge N, van der Luijt RB, Meijers-Heijboer H, Gómez García EB; HEBON, Devilee P, Vreeswijk MP, Lubinski J, Jakubowska A, Gronwald J, Huzarski T, Byrski T, Górski B, Cybulski C, Spurdle AB, Holland H; kConFab, Goldgar DE, John EM, Hopper JL, Southey M, Buys SS, Daly MB, Terry MB, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Preisler-Adams S, Arnold N, Niederacher D, Sutter C, Domchek SM, Nathanson KL, Rebbeck T, Blum JL, Piedmonte M, Rodriguez GC, Wakeley K, Boggess JF, Basil J, Blank SV, Friedman E, Kaufman B, Laitman Y, Milgrom R, Andrulis IL, Glendon G, Ozcelik H, Kirchhoff T, Vijai J, Gaudet MM, Altshuler D, Guiducci C; SWE-BRCA, Loman N, Harbst K, Rantala J, Ehrencrona H, Gerdes AM, Thomassen M,

Sunde L, Peterlongo P, Manoukian S, Bonanni B, Viel A, Radice P, Caldes T, de la Hoya M, Singer CF, Fink-Retter A, Greene MH, Mai PL, Loud JT, Guidugli L, Lindor NM, Hansen TV, Nielsen FC, Blanco I, Lazaro C, Garber J, Ramus SJ, Gayther SA, Phelan C, Narod S, Szabo CI; MOD SQUAD, Benitez J, Osorio A, Nevanlinna H, Heikkinen T, Caligo MA, Beattie MS, Hamann U, Godwin AK, Montagna M, Casella C, Neuhausen SL, Karlan BY, Tung N, Toland AE, Weitzel J, Olopade O, Simard J, Soucy P, Rubinstein WS, Arason A, Rennert G, Martin NG, Montgomery GW, Chang-Claude J, Flesch-Janys D, Brauch H; GENICA, Severi G, Baglietto L, Cox A, Cross SS, Miron P, Gerty SM, Tapper W, Yannoukakos D, Fountzilas G, Fasching PA, Beckmann MW, Dos Santos Silva I, Peto J, Lambrechts D, Paridaens R, Rüdiger T, Försti A, Winqvist R, Pylkäs K, Diasio RB, Lee AM, Eckel-Passow J, Vachon C, Blows F, Driver K, Dunning A, Pharoah PP, Offit K, Pankratz VS, Hakonarson H, Chenevix-Trench G, Easton DF, Couch FJ. A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet 2010;42:885-92.

- Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, Zavoral M, Lechuga MJ, Gerletti P, Tang J, *et al*. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med 2006;355:885–95.
- Armstrong K, Eisen A, Weber B. Assessing the risk of breast cancer. N Engl J Med 2000;342:564-71.
- Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770-6.
- Arteaga CL, Dugger TC, Hurd SD. The multifunctional role of transforming growth factor (TGF)-beta s on mammary epithelial cell biology. Breast Cancer Res Treat 1996;38:49-56.
- Asaga S, Jinno H, Sakata M, Kitajima M. Current perspective of chemoprevention in breast cancer. Nihon Rinsho 2007;65:627-32.
- Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast 2011;20:66-70.
- Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, Milne GL, Katkuri S, DuBois RN. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem 2005;280:3217–23.
- Bagnardi V, Rota M, Botteri E, et al. Light alcohol drinking and cancer: a meta-analysis. Ann Oncol 2013; 24:301.
- Bakken K, Fournier A, Lund E, *et al*. Menopausal hormone therapy and breast cancer risk: impact of different treatments. The European Prospective Investigation into Cancer and Nutrition. Int J Cancer 2011;128:144.
- Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539-45.
- Bardia A, Ebbert JO, Vierkant RA, Limburg PJ, Anderson K, Wang AH, Olson JE, Vachon CM, Cerhan JR. Association of aspirin and nonaspirin nonsteroidal antiinflammatory drugs with cancer incidence and mortality. J Natl Cancer Inst 2007;99:881-9.
- Bardia A, Olson JE, Vachon CM, Lazovich D, Vierkant RA, Wang AH, Limburg PJ, Anderson KE, Cerhan JR. Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: results from a prospective cohort study. Breast Cancer Res Treat 2011;126:149-55.

- Barnes N, Haywood P, Flint P, Knox WF, Bundred NJ. Survivin expression in in situ and invasive breast cancer relates to COX-2 expression and DCIS recurrence. Br J Cancer 2006; 94: 253-8 .
- Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, Bolognese JA, Oxenius B, Horgan K, Loftus S, *et al*. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology 2006;131:1674–82.
- Baron JA, Sandler RS. Nonsteroidal antiinflammatory drugs and cancer prevention. Annu Rev Med 2000;51:511–23.
- Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, McNabb MA, Wenger NK, Raloxifene Use for The Heart (RUTH) Trial Investigators. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med 2006;355:125-37.
- Basu GD, Pathangey LB, Tinder TL, Lagioia M, Gendler SJ, Mukherjee P. Cyclooxygenase-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer. Mol Cancer Res 2004;2:632-42.
- Beatson GT: On treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. Lancet 1896;148:104–7.
- Beattie MS, Costantino JP, Cummings SR, *et al*. Endogenous sex hormones, breast cancer risk, and tamoxifen response: an ancillary study in the NSABP Breast Cancer Prevention Trial (P-1). J Natl Cancer Inst 2006;98110.
- Benoit V, Relic B, Leval Xd Xd, Chariot A, Merville MP, Bours V. Regulation of HER-2 oncogene expression by cyclooxygenase-2 and prostaglandin E2. Oncogene 2004;23:1631-1635. Quitar si al final quito ese párrafo sobre el pronóstico de HER2
- Beral V and Million Women Study Coordinators. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 2003;362:419-27
- Beral V, Reeves G, Bull D, *et al*. Breast cancer risk in relation to the interval between menopause and starting hormone therapy. J Natl Cancer Inst 2011;103:296.
- Berinder K, Akre O, Granath F, Hulting AL. Cancer risk in hyperprolactinemia patients: a population-based cohort study. Eur J Endocrinol 2011;165:209-15
- Bernier MO, Plu-Bureau G, Bossard N, Ayzac L, Thalabard JC. Breastfeeding and risk of breast cancer: a metaanalysis of published studies. Hum Reprod Update 2000;6:374-86.
- Bernstein L, Henderson BE, Hanisch R, Sullivan-Halley J, Ross RK. Physical exercise and reduced risk of breast cancer in young women. J Natl Cancer Inst 1994;86:1403-8.
- Bernstein L, Ross RK. Endogenous hormones and breast cancer risk. Epidemiol Rev 1993; 15:48-65.
- Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, Tang J, Rosenstein RB, Wittes J, Corle D, *et al*. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med 2006;355:873–84.
- Bevan JS, Atkin SL, Atkinson AB, Bouloux PM, Hanna F, Harris PE, *et al*. Primary medical therapy for acromegaly: an open, prospective, multicenter study of the effects of subcutaneous and

intramuscular slowrelease octreotide on growth hormone, insulin-like growth factor-I, and tumor size. J Clin Endocrinol Metab 2002;87:4554-63.

- Bewtra C, Watson P, Conway T, Read-Hippee C, Lynch HT. Hereditary ovarian cancer: a clinicopathological study. Int J Gynecol Pathol 1992;11:180-7.
- Bhattacharjee RN, Timoshenko AV, Cai J, Lala PK. Rela- tionship between cyclooxygenase-2 and human epidermal growth factor receptor 2 in vascular endothelial growth fac- tor C up-regulation and lymphangiogenesis in human breast cancer. Cancer Sci 2010;101:2026-2032
- Boardman LA, Thibodeau SN, Schaid DJ, *et al*. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998;128:896-9.
- Bocca C, Ievolella M, Autelli R, Motta M, Mosso L, Torchio B, Bozzo F, Cannito S, Paternostro C, Colombatto S, Parola M, Miglietta A. Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. Expert Opin Ther Targets 2014;18:121-35.
- Boland GP, Butt IS, Prasad R, Knox WF, Bundred NJ. COX-2 expression is associated with an aggressive phenotype in ductal carcinoma in situ. Br J Cancer 2004;90:423-29.
- Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ, Group VIGORS. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med. 2000;343:1520–28.
- Bontenbal M, Foekens JA, Lamberts SW, de Jong FH, van Putten WL, Braun HJ, *et al*. Feasibility, endocrine and anti-tumour effects of a triple endocrine therapy with tamoxifen, a somatostatin analogue and an antiprolactin in post-menopausal metastatic breast cancer: a randomized study with long-term follow-up. Br J Cancer 1998;77:115-22.
- Bosco JL, Palmer JR, Boggs DA, Hatch EE, Rosenberg L. Regular aspirin use and breast cancer risk in US Black women. Cancer Causes Control 2011;2:1553-61.
- Bosetti C, Gallus S, La Vecchia C. Aspirin and cancer risk: an updated quantitative review to 2005. Cancer Causes Control 2006;17:871-88.
- Bosetti C, Rosato V, Gallus S, Cuzick J, La Vecchia C. Aspirin and cancer risk: a quantitative review to 2011.Ann Oncol 2012;23:1403-15.
- Bousquet C, Puente E, Buscail L, Vaysse N, Susini C. Antiproliferative effect of somatostatin and analogs. Chemotherapy 2001;47:30-9.
- Boyd NF, Dite GS, Stone J, *et al*. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347:886-94.
- Boyd NF, Greenberg C, Lockwood G, *et al*. Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. Canadian Diet and Breast Cancer Prevention Study Group. J Natl Cancer Inst 1997;89:488-96.
- Boyd NF, Guo H, Martin LJ, *et al*. Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356:227.
- Boyd NF, Rommens JM, Vogt K, *et al*. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 2005;6:798-808.

- Bracke ME, Charlier C, Bruyneel EA, Labit C, Mareel MM, Castronovo V. Tamoxifen restores the E-cadherin function in human breast cancer MCF-7/6 cells and suppresses their invasive phenotype. Cancer Res 1994;54(17):4607-9.
- Brasky TM, Bonner MR, Moysich KB, Ambrosone CB, Nie J, Tao MH, Edge SB, Kallakury BV, Marian C, Trevisan M, Shields PG, Freudenheim JL. Non-steroidal antiinflammatory drug (NSAID) use and breast cancer risk in the Western New York Exposures and Breast Cancer (WEB) Study. Cancer Causes Control. 2010;21:1503-12.
- Brasky TM, Bonner MR, Moysich KB, Ambrosone CB, Nie J, Tao MH, Edge SB, Kallakury BV, Marian C, Goerlitz DS, Trevisan M, Shields PG, Freudenheim JL. Non-steroidal antiinflammatory drugs (NSAIDs) and breast cancer risk: differences by molecular subtype. Cancer Causes Control 2011;22:965-75.
- Brasky TM, Bonner MR, Moysich KB, Ochs-Balcom HM, Marian C, Ambrosone CB, Nie J, Tao MH, Edge SB, Trevisan M, Shields PG, Freudenheim JL. Genetic variants in COX-2, non-steroidal antiinflammatory drugs, and breast cancer risk: the Western New York Exposures and Breast Cancer (WEB) Study. Breast Cancer Res Treat 2011;126:157-65.
- Braun R, Buetow K. Pathways of distinction analysis: a new technique for multi-SNP analysis of GWAS data. PLoS Genet 2011;7:e1002101.
- Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1996;347:1713-27.
- Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1997; 350:1047.
- Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst 2006 Oct 4;98(19):1382-96.
- Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, *et al*. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352:1092–102.
- Brettes JP, Mathelin C. Dual effects of androgens on mammary gland. Bull Cancer 2008;95:495-502.
- Brinton LA, Schairer C, Hoover RN, Fraumeni JF Jr. Menstrual factors and risk of breast cancer. Cancer Invest 1988;6:245-54.
- Brockwell SE, Gordon IR. A comparison of statistical methods for metaanalysis. StatMed 2001;20:825–40.
- Brodie AM, Lu Q, Long BJ, Fulton A, Chen T, Macpherson N, DeJong PC, Blankenstein MA, Nortier JW, Slee PH, *et al*. Aromatase and COX-2 expression in human breast cancers. J Steroid Biochem Mol Biol 2001;79:41–7.

- Brown JR, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. J Clin Oncol 2005;23:2840–55.
- Brueggemeier RW, Diaz-Cruz ES, Li PK, Sugimoto Y, Lin YC, Shapiro CL. Translational studies on aromatase, cyclooxygenases, and enzyme inhibitors in breast cancer. Journal of Steroid Biochemistry & Molecular Biology 2005;95:129–36.
- Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, Robertson FM. Correlation of aromatase and cycloox- ygenase gene expression in human breast cancer specimens. Cancer Lett 1999;140:27-35.
- Bruzzi P, Negri E, La Vecchia C, Decarli A, Palli D, Parazzini F, Del Turco MR. Short term increase in risk of breast cancer after full term pregnancy. BMJ 1988;297:1096-8.
- Bulun SE, Lin Z, Imir G, *et al*. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. Pharmacol Rev 2005;57:359-83.
- Burd R, Choy H, Dicker A. Potential for inhibitors of cyclo- oxygenase-2 to enhance tumor radioresponse. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Hu- mana Press, Totowa, NJ, 2002:301-311
- Buttyan R, Sawczuk IS, Benson MC, Siegal JD, Olsson CA. Enhanced expression of the c-myc protooncogene in high-grade human prostate cancers. Prostate 1987;11:327-37.
- Camp NJ, Parry M, Knight S, Abo R, Elliott G, Rigas SH, Balasubramanian SP, Reed MW, McBurney H, Latif A, Newman WG, Cannon-Albright LA, Evans DG, Cox A. Fine-mapping CASP8 risk variants in breast cancer. Cancer Epidemiol Biomarkers Prev 2012;21:176-81.
- Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD, Buring JE, Chanock SJ, Diver WR, Dostal L, Fournier A, Hankinson SE, Henderson BE, Hoover RN, Isaacs C, Johansson M, Kolonel LN, Kraft P, Lee IM, McCarty CA, Overvad K, Panico S, Peeters PH, Riboli E, Sanchez MJ, Schumacher FR, Skeie G, Stram DO, Thun MJ, Trichopoulos D, Zhang S, Ziegler RG, Hunter DJ, Lindström S, Canzian F. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. J Natl Cancer Inst 2011;103:1252-63.
- Cantor S, Drapkin R, Zhang F, Lin Y, Han J, Pamidi S, Livingston DM. The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. Proc Natl Acad Sci U S A. 2004;101:2357-62.
- Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 2001;105:149-60.
- Cappellani A, Vita MD, Zanghì A, *et al*. Prognostic factors in elderly patients withbreast cancer. BMC Surgery 2013;13:S2
- Carter CA, Milholland RJ, Shea W, Ip MM. Effect of the prostaglandin synthetase inhibitor indomethacin on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in rats fed different levels of fat. Cancer Res 1983;433559-62.
- Castellone MD, Teramoto H, Williams BO, Druey KM, Gut- kind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science 2005;310: 1504-10

- Cauley JA, Lucas FL, Kuller LH, *et al*. Bone mineral density and risk of breast cancer in older women: the study of osteoporotic fractures. Study of Osteoporotic Fractures Research Group. JAMA 1996;276:1404.
- Cauley JA, Lucas FL, Kuller LH, *et al*. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group. Ann Intern Med 1999;130:270.
- Cavalieri EL, Rogan EG, Chakravarti D. Initiation of cancer and other diseases by catechol ortho-quinones: a unify- ing mechanism. Cell Mol Life Sci 2002;59:665-681
- Cedro-Tanda A, Córdova-Solis A, Juárez-Cedillo T, Pina-Jiménez E, Hernández-Caballero ME, Moctezuma-Meza C, Castelazo-Rico G, Gómez-Delgado A, Monsalvo-Reyes AC, Salamanca-Gómez FA, Arenas-Aranda DJ, García-Hernández N. Prevalence of HMTV in breast carcinomas and unaffected tissue from Mexican women. BMC Cancer 2014;14:942.
- Centro Nacional de Epidemiología. Mortalidad por cáncer y otras causas en España, 2013. Área de Epidemiología Ambiental, Centro Nacional de Epidemiología, ISCIII. http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/fd-epidemiologia-ambiental-y-cancer/Mortal2013.pdf (accessed: June 3, 2015).
- Chan AT, Manson JE, Feskanich D, Stampfer MJ, Colditz GA, Fuchs CS. Long-term aspirin use and mortality in women. Arch Intern Med 2007;167:562-72.
- Chang SH, Liu CH, Conway R, Han DK, Nithipatikom K, Trifan OC, Lane TF, Hla T. Role of prostaglandin E_2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. Proc Natl Acad Sci USA 2004;101:591–6.
- CHEK2 Breast Cancer Case-Control Consortium. CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 2004;74:1175-82.
- Chen MB, Li C, Shen WX, Guo YJ, Shen W, Lu PH.Association of a LSP1 gene rs3817198T>C polymorphism with breast cancer risk: evidence from 33,920 cases and 35,671 controls. Mol Biol Rep 2011;38:4687-95.
- Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 2007;25:1329-33.
- Chen S, Zhou D, Okubo T, Kao YC, Yang C. Breast tumor aromatase: functional role and transcriptional regulation. Endocr Relat Cancer 1999;6:149–56.
- Chen WY, Colditz GA, Rosner B, et al. Use of postmenopausal hormones, alcohol, and risk for invasive breast cancer. Ann Intern Med 2002;137:798.
- Chen WY, Manson JE, Hankinson SE, Rosner B, Holmes MD, Willett MC, *et al*. Unopposed estrogen therapy and the risk of invasive breast cancer. Arch Intern Med 2006;166:1027-32
- Chen WY, Rosner B, Hankinson SE, et al. Moderate alcohol consumption during adult life, drinking patterns, and breast cancer risk. JAMA 2011;306:1884.
- Chen WY. Epidemiology and risk factors for breast cancer. In: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA. (Accesed on February 14th, 2015).

- Chen Y, Thompson W, Semenciw R, Mao Y. Epidemiology of contralateral breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:855-61.
- Chen Z, Arendell L, Aickin M, *et al*. Hip bone density predicts breast cancer risk independently of Gail score: results from the Women's Health Initiative. Cancer 2008;113:907.
- Chlebowski RT, Anderson GL, Gass M, *et al*. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. JAMA 2010;304:1684.
- Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, *et al*. Influence of estrogen plus progestin on breast cancer and mammography in healty postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA 2003;289:3243-53.
- Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, Aragaki AK, Ockene JK, Lane DS, Sarto GE, Rajkovic A, Schenken R, Hendrix SL, Ravdin PM, Rohan TE, Yasmeen S, Anderson G; WHI Investigators. Breast cáncer after use of estrogen plus progestin in postmenopausal women. N Engl J Med 2009;360:573-87.
- Choumenkovitch SF, Selhub J, Wilson PW, et al. Folic acid intake from fortification in United States exceeds predictions. J Nutr 2002;132:2792.
- Chow LW, Zhu L, Loo WT, Lui EL. Aberrant methylation of cyclooxygenase-2 in breast cancer patients. Biomed Pharmacother 2005;59:S264-S267.
- Chuah BY, Putti T, Salto-Tellez M, Charlton A, Iau P, Buhari SA, Wong CI, Tan SH, Wong AL, Chan CW, Goh BC, Lee SC. Serial changes in the expression of breast cancer-related proteins in response to neoadjuvant chemotherapy. Ann Oncol 2011;22:1748-54 .
- Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, Tiano HF, Morham SG, Smithies O, Langenbach R. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res 2000;60:4705-8.
- Chung S, Park SK, Sung H, Song N, Han W, Noh DY, Ahn SH, Yoo KY, Choi JY, Kang D. Association between chronological change of reproductive factors and breast cancer risk defined by hormone receptor status: results from the Seoul Breast Cancer Study. Breast Cancer Res Treat 2013;140:557-65.
- Clària J. Cyclooxygenase-2 biology. Curr Pharm Des 2003;9:2177-90
- Clarke C, Sutherland R. Progestin regulation of cellular proliferation: Update 1993. In: Endocrine Reviews, Horwitz K (Ed), Endocrine Society, Bethesda, MD 1993. p.132.
- Clavel-Chapelon F, E3N Group. Cumulative number of menstrual cycles and breast cancer risk: results from the E3N cohort study of French women. Cancer Causes Control 2002;13:831-8.
- Clevers H. Colon cancer - understanding how NSAIDs work. N Engl J Med 2006;354:761-3.
- Cline JM, Soderqvist G, von Schoultz E, *et al*. Effects of hormone replacement therapy on the mammary gland of surgically postmenopausal cynomolgus macaques. Am J Obstet Gynecol 1996;174:93.
- Coffey RJ, Hawkey CJ, Damstrup L, Graves-Deal R, Daniel VC, Dempsey PJ, Chinery R, Kirkland SC, DuBois RN, Jetton TL, Morrow JD. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenesis in polarizing colon cancer cells. Proc Natl Acad Sci USA 1997;94:657-62.

- Colditz GA, Frazier AL. Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. Cancer Epidemiol Biomarkers Prev 1995;4:567-71.
- Colditz GA, Rosner B. Cumulative risk of breast cancer to age 70 years according to risk factor status: data from the Nurses' Health Study. Am J Epidemiol 2000;152:950-64.
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002;360:187.
- Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1997;350:1047-59
- Coogan PF, Rao SR, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD, Shapiro S. The relationship of nonsteroidal antiinflammatory drug use to the risk of breast cancer. Prev Med 1999;29:72-6.
- Coogan PF, Rosenberg L, Louik C, Zauber AG, Stolley PD, Strom BL, Shapiro S. NSAIDs and risk of colorectal cancer according to presence or absence of family history of the disease. Cancer Causes Control 2000;11:249–55.
- Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. JAMA 2005;294:47-55.
- Cordina-Duverger E, Truong T, Anger A, Sanchez M, Arveux P, Kerbrat P, Guénel P. Risk of breast cancer by type of menopausal hormone therapy: a case-control study among post-menopausal women in France. PLoS One 2013;8:e78016.
- Cornejo-Moreno BA, Uribe-Escamilla D, Salamanca-Gómez F. Breast cancer genes: looking for BRACA's lost brother. Isr Med Assoc J 2014;16:787-92.
- Corpet DE, Pierre F. Point: from animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. Cancer Epidemiol Biomarkers Prev 2003;12:391–400.
- Costa C, Soares R, Reis-Filho JS, Leitao D, Amendoeira I, Schmitt FC. Cyclo-oxygenase-2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. J Clin Pathol 2002;55:429–34.
- Cotterchio M, Kreiger N, Sloan M, Steingart A. Nonsteroidal antiinflammatory drug use and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:1213-7.
- Couch FJ, DeShano ML, Blackwood MA, *et al*. BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. N Engl J Med 1997;336:1409-15.
- Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.
- Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, Pooley KA, Scollen S, Baynes C, Ponder BA, Chanock S, Lissowska J, Brinton L, Peplonska B, Southey MC, Hopper JL, McCredie MR, Giles GG, Fletcher O, Johnson N, dos Santos Silva I, Gibson L, Bojesen SE, Nordestgaard BG, Axelsson CK, Torres D, Hamann U, Justenhoven C, Brauch H, Chang-Claude J,

Kropp S, Risch A, Wang-Gohrke S, Schürmann P, Bogdanova N, Dörk T, Fagerholm R, Aaltonen K, Blomqvist C, Nevanlinna H, Seal S, Renwick A, Stratton MR, Rahman N, Sangrajrang S, Hughes D, Odefrey F, Brennan P, Spurdle AB, Chenevix-Trench G; Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer, Beesley J, Mannermaa A, Hartikainen J, Kataja V, Kosma VM, Couch FJ, Olson JE, Goode EL, Broeks A, Schmidt MK, Hogervorst FB, Van't Veer LJ, Kang D, Yoo KY, Noh DY, Ahn SH, Wedrén S, Hall P, Low YL, Liu J, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Sigurdson AJ, Stredrick DL, Alexander BH, Struewing JP, Pharoah PD, Easton DF; Breast Cancer Association Consortium. A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 2007;39:352-8.

- Cramer DW, Harlow BL, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. Over-the-counter analgesics and risk of ovarian cancer. Lancet 1998;351:104–7.
- Crawford YG, Gauthier ML, Joubel A, Mantei K, Kozakiewicz K, Afshari CA, Tlsty TD. Histologically normal human mammary epithelia with silenced p16(INK4a) overexpress COX-2, promoting a premalignant program. Cancer Cell 2004;5:263–73.
- Cronin-Fenton DP, Pedersen L, Lash TL, Friis S, Baron JA, Sørensen HT. Prescriptions for selective cyclooxygenase-2 inhibitors, non-selective non-steroidal antiinflammatory drugs, and risk of breast cancer in a population-based case-control study. Breast Cancer Res 2010;12:R15.
- Cummings SR, Tice JA, Bauer S, *et al*. Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. J Natl Cancer Inst 2009;101:384.
- Cuzick J, Forbes J, Edwards R, Baum M, Cawthorn S, Coates A, *et al*. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. Lancet 2002;360:817-24.
- Cuzick J, Sestak I, Cawthorn S, Hamed H, Holli K, Howell A, Forbes JF; IBIS-I Investigators. Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial. Lancet Oncol 2015;16:67-75.
- Cuzick J, Sestak I, Forbes JF, Dowsett M, Knox J, Cawthorn S, Saunders C, Roche N, Mansel RE, von Minckwitz G, Bonanni B, Palva T, Howell A; IBIS-II investigators. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomized placebo-controlled trial. Lancet 2014;383:1041-8.
- Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB, Subbaramaiah K. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. Lancet Oncol 2001;2:544–51.
- Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. J Clin Oncol 2005;23:254-66
- Davidson NE, Helzlsouer KJ. Good news about oral contraceptives. N Engl J Med 2002;346:2078.
- Davies G, Salter J, Hills M, Martin LA, Sacks N, Dowsett M. Correlation between cyclooxygenase-2 expression and angiogenesis in human breast cancer. Clin Cancer Res 2003;9:2651-6
- Davis S, Mirick DK. Medication use and the risk of breast cancer. Eur J Epidemiol 2007;22:319-25.

- Dawood S. Triple-negative breast cancer: epidemiology and management options. Drugs 2010;70:2247-58.
- Daye D, Keller B, Conant EF, Chen J, Schnall MD, Maidment AD, Kontos D. Mammographic parenchymal patterns as an imaging marker of endogenous hormonal exposure: a preliminary study in a high-risk population. Acad Radiol 2013;20:635-46.
- de Jong MM, Nolte IM, de Vries EG, *et al*. The HLA class III subregion is responsible for an increased breast cancer risk. Hum Mol Genet 2003; 12:2311-9.
- De Leeneer K, Van Bockstal M, De Brouwer S, Swietek N, Schietecatte P, Sabbaghian N, Van den Ende J, Willocx S, Storm K, Blaumeiser B, Van Asperen CJ, Wijnen JT, Leunen K, Legius E, Michils G, Matthijs G, Blok MJ, Gomez-Garcia E, De Paepe A, Tischkowitz M, Poppe B, Claes K. Evaluation of RAD51C as cancer susceptibility gene in a large breast-ovarian cancer patient population referred for genetic testing. Breast Cancer Res Treat 2012;133:393-8.
- Deb S, Wong SQ, Li J, Do H, Weiss J, Byrne D, Chakrabarti A, Bosma T; kConFab Investigators, Fellowes A, Dobrovic A, Fox SB. Mutational profiling of familial male breast cancers reveals similarities with luminal A female breast cancer with rare TP53 mutations. Br J Cancer. 2014;111:2351-60
- Deligdisch L, Marin T, Lee AT, Etkind P, Holland JF, Melana S, Pogo BG. Human mammary tumor virus (HMTV) in endometrial carcinoma. Int J Gynecol Cancer 2013;23:1423-8.
- DeMichele A, Troxel AB, Berlin JA, Weber AL, Bunin GR, Turzo E, Schinnar R, Burgh D, Berlin M, Rubin SC, Rebbeck TR, Strom BL. Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case–control study. J Clin Oncol 2008;26:4151–9.
- Denkert C, Winzer KJ, Müller BM, Weichert W, Pest S, Köbel M, Kristiansen G, Reles A, Siegert A, Guski H, Hauptmann S. Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer 2003;97:2978-87.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
- DeSantis C, Siegel R, Bandi P, Jemal A. Breast cancer statistics, 2011. CA Cancer J Clin 2011;61:409-18.
- Díaz-Cruz ES, Brueggemeier RW. Interrelationships be- tween cyclooxygenases and aromatase: unraveling the relevance of cyclooxygenase inhibitors in breast cancer. An- ticancer Agents Med Chem 2006;6:221-32.
- Diaz-Cruz ES, Shapiro CL, Brueggemeier RW. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. J Clin Endocrinol Metab 2005;90:2563–70.
- Díez-Gilbert O. Estudio molecular de los genes BRCA1 y BRCA2 en cáncer de mama hereditario. Ed Cont Lab Clín 2006;9:19-27.
- Ding Y, Tong M, Liu S, Moscow JA, Tai HH. NAD+/-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. Carcinogenesis 2005;26:65–72.
- Dolan JT, Miltenburg DM, Granchi TS, Miller CC 3rd, Brunicardi FC. Treatment of metastatic breast cancer with somatostatin analogues–a meta-analysis. Ann Surg Oncol 2001;8:227-33.

- Domcheck SM, Stopfer JE, Rebbeck TR. Bilateral Risk-Reducing Oophorectomy in BRCA1 and BRCA2 Mutation Carriers. J Natl Compr Canc Netw 2006;4:177-82.
- Dorgan JF, Stanczyk FZ, Kahle LL, Brinton LA. Prospective casecontrol study of premenopausal serum estradiol and testosterone levels and breast cancer risk. Breast Cancer Res 2010; 12:R98.
- Dubé C, Rostom A, Lewin G, Tsertsvadze A, Barrowman N, Code C, Sampson M, Moher D; U.S. Preventive Services Task Force. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med 2007;146:365-75.
- Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063-73.
- Dumas I, Diorio C. Estrogen pathway polymorphisms and mammographic density. Anticancer Res 2011;31:4369-86.
- Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC. A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 2003;63:2610-5.
- Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000;56:455-63
- Easton DF, Bishop DT, Ford D, Crockford GP. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1993b;52:678-701.
- Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R; SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schürmann P, Dörk T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X; kConFab; AOCS Management Group, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087-93.
- Egan KM, Stampfer MJ, Giovannucci E, Rosner BA, Colditz GA. Prospective study of regular aspirin use and the risk of breast cancer. J Natl Cancer Inst 1996;88:988-93.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Brit Med J 1997;315:629-34.
- Eisen A, Lubinski J, Klijn J, Moller P, Lynch HT, Offit K, Weber B, Rebbeck T, Neuhausen SL, Ghadirian P, Foulkes WD, Gershoni-Baruch R, Friedman E, Rennert G, Wagner T, Isaacs C, Kim-

Sing C, Ainsworth P, Sun P, Narod SA. Breast cancer risk following bilateral oophorectomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. J Clin Oncol 2005;23:7491-6

- Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. Use of aspirin, other nonsteroidal antiinflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. Arch Intern Med. 2009;169(2):115-21;
- Eliassen AH, Colditz GA, Rosner B *et al*. Adult weight change and risk of postmenopausal breast cancer. JAMA 2006;296:193.
- Eliassen AH, Missmer SA, Tworoger SS, *et al*. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. J Natl Cancer Inst 2006;98:1406.
- Ellem SJ, Risbridger GP. Aromatase and prostate cancer. Minerva Endocrinol 2006;31:1-12
- Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, *et al*. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol 2010;11:530.
- Entrez Gene: RAD51L1 RAD51-like 1 (S. cerevisiae).
- Erlandsson G, Montgomery SM, Cnattingius S, Ekbom A. Abortions and breast cancer: record-based case-control study. Int J Cancer 2003; 103:676.
- Eroles P, Bosch A, Pérez-Fidalgo JA, Lluch A. Molecular biology in breast cancer: Intrinsic subtypes and signaling pathways. Cancer Treatment Rev 2012;38:698-707.
- Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, Gabrielson E, Schutte M, Baylin SB, Herman JG. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 2000;92:564-9.
- Fachal L, Dunning AM. From candidate gene studies to GWAS and post-GWAS analyses in breast cancer. Curr Opin Genet Dev 2015;30C:32-41.
- Falk RT, Maas P, Schairer C, et al. Alcohol and risk of breast cancer in postmenopausal women: an analysis of etiological heterogeneity by multiple tumor characteristics. Am J Epidemiol 2014;180:705.
- Farhat GN, Cummings SR, Chlebowski RT *et al*. Sex hormone levels and risks of estrogen receptor-negative and estrogen receptor-positive breast cancers. J Natl Cancer Inst 2011; 103:562.
- Farrow DC, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF, Jr, Blot WJ. Use of aspirin and other nonsteroidal antiinflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 1998;7:97–102.
- Fasching PA, Pharoah PD, Cox A, Nevanlinna H, Bojesen SE, Karn T, Broeks A, van Leeuwen FE, van 't Veer LJ, Udo R, Dunning AM, Greco D, Aittomäki K, Blomqvist C, Shah M, Nordestgaard BG, Flyger H, Hopper JL, Southey MC, Apicella C, Garcia-Closas M, Sherman M, Lissowska J, Seynaeve C, Huijts PE, Tollenaar RA, Ziogas A, Ekici AB, Rauh C, Mannermaa A, Kataja V, Kosma VM, Hartikainen JM, Andrulis IL, Ozcelik H, Mulligan AM, Glendon G, Hall P, Czene K, Liu J, Chang-Claude J, Wang-Gohrke S, Eilber U, Nickels S, Dörk T, Schiekel M, Bremer M, Park-Simon TW, Giles GG, Severi G, Baglietto L, Hooning MJ, Martens JW, Jager A, Kriege M, Lindblom A,

Margolin S, Couch FJ, Stevens KN, Olson JE, Kosel M, Cross SS, Balasubramanian SP, Reed MW, Miron A, John EM, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Kauppila S, Burwinkel B, Marme F, Schneeweiss A, Sohn C, Chenevix-Trench G, kConFab Investigators, Lambrechts D, Dieudonne AS, Hatse S, van Limbergen E, Benitez J, Milne RL, Zamora MP, Pérez JI, Bonanni B, Peissel B, Loris B, Peterlongo P, Rajaraman P, Schonfeld SJ, Anton-Culver H, Devilee P, Beckmann MW, Slamon DJ, Phillips KA, Figueroa JD, Humphreys MK, Easton DF, Schmidt MK. The role of genetic breast cancer susceptibility variants as prognostic factors. Hum Mol Genet 2012;21:3926-39.

- Feigelson HS, Jonas CR, Teras LR, *et al*. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2004;13:220.
- Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374-403.
- Figueroa JD, Garcia-Closas M, Humphreys M, Platte R, Hopper JL, Southey MC, Apicella C, Hammet F, Schmidt MK, Broeks A, Tollenaar RA, Van't Veer LJ, Fasching PA, Beckmann MW, Ekici AB, Strick R, Peto J, dos Santos Silva I, Fletcher O, Johnson N, Sawyer E, Tomlinson I, Kerin M, Burwinkel B, Marme F, Schneeweiss A, Sohn C, Bojesen S, Flyger H, Nordestgaard BG, Benítez J, Milne RL, Ignacio Arias J, Zamora MP, Brenner H, Müller H, Arndt V, Rahman N, Turnbull C, Seal S, Renwick A, Brauch H, Justenhoven C, Brüning T; GENICA Network, Chang-Claude J, Hein R, Wang-Gohrke S, Dörk T, Schürmann P, Bremer M, Hillemanns P, Nevanlinna H, Heikkinen T, Aittomäki K, Blomqvist C, Bogdanova N, Antonenkova N, Rogov YI, Karstens JH, Bermisheva M, Prokofieva D, Gantcev SH, Khusnutdinova E, Lindblom A, Margolin S, Chenevix-Trench G, Beesley J, Chen X; kConFab AOCS Management Group, Mannermaa A, Kosma VM, Soini Y, Kataja V, Lambrechts D, Yesilyurt BT, Chrisiaens MR, Peeters S, Radice P, Peterlongo P, Manoukian S, Barile M, Couch F, Lee AM, Diasio R, Wang X, Giles GG, Severi G, Baglietto L, Maclean C, Offit K, Robson M, Joseph V, Gaudet M, John EM, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Grip M, Andrulis I, Knight JA, Mulligan AM, O'Malley FP, Brinton LA, Sherman ME, Lissowska J, Chanock SJ, Hooning M, Martens JW, van den Ouweland AM, Collée JM, Hall P, Czene K, Cox A, Brock IW, Reed MW, Cross SS, Pharoah P, Dunning AM, Kang D, Yoo KY, Noh DY, Ahn SH, Jakubowska A, Lubinski J, Jaworska K, Durda K, Sangrajrang S, Gaborieau V, Brennan P, McKay J, Shen CY, Ding SL, Hsu HM, Yu JC, Anton-Culver H, Ziogas A, Ashworth A, Swerdlow A, Jones M, Orr N, Trentham-Dietz A, Egan K, Newcomb P, Titus-Ernstoff L, Easton D, Spurdle AB. Associations of common variants at 1p11.2 and 14q24.1 (RAD51L1) with breast cancer risk and heterogeneity by tumor subtype: findings from the Breast Cancer Association Consortium. Hum Mol Genet 2011;20:4693-706.
- Finch A, Beiner M, Lubinski J, *et al.* . Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 mutation. JAMA. 2006;296:185-92.
- Fiorelli G, Picariello L, Martineti V, Tonelli F, Brandi ML. Estrogen synthesis in human colon cancer epithelial cells. J Steroid Biochem Mol Biol 1999;71:223-30.
- Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; 90:1371-88.

- Fletcher O, Johnson N, Dos Santos Silva I, Kilpivaara O, Aittomäki K, Blomqvist C, Nevanlinna H, Wasielewski M, Meijers-Heijerboer H, Broeks A, Schmidt MK, Van't Veer LJ, Bremer M, Dörk T, Chekmariova EV, Sokolenko AP, Imyanitov EN, Hamann U, Rashid MU, Brauch H, Justenhoven C, Ashworth A, Peto J. Family history, genetic testing, and clinical risk prediction: pooled analysis of CHEK2 1100del in 1,828 bilateral breast cancers and 7,030 controls. Cancer Epidemiol Biomarkers Prev 2009;18:230-4.
- Fletcher O, Johnson N, Orr N, Hosking FJ, Gibson LJ, Walker K, Zelenika D, Gut I, Heath S, Palles C, Coupland B, Broderick P, Schoemaker M, Jones M, Williamson J, Chilcott-Burns S, Tomczyk K, Simpson G, Jacobs KB, Chanock SJ, Hunter DJ, Tomlinson IP, Swerdlow A, Ashworth A, Ross G, dos Santos Silva I, Lathrop M, Houlston RS, Peto J. Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. J Natl Cancer Inst. 2011;103:425-35.
- Folkman J. Angiogenesis. Annu Rev Med 2006;57:1-18.
- Forman D, Bray F, Brewster DH, Gombe Mbalawa C, Kohler B, Piñeros M, Steliarova-Foucher E, Swaminathan R and Ferlay J, editors (2013). Cancer Incidence in Five Continents, Vol. X (electronic version). Lyon: International Agency for Research on Cancer. Available from: http://ci5.iarc.fr, accessed [05/18/15]
- Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat 2008;107:103.
- Fournier A, Berrino F, Riboli E *et al*. Breast cancer risk in relation to different types of hormone replacement therapy in the E3N-EPIC cohort. Int J Cancer 2005;114:448.
- Frati A, Antoine M, Rodenas A, Gligorov J, Rouzier R, Chéreau E. Somatostatin in breast cancer. Ann Biol Clin 2011;69:385-91.
- Friebel TM, Domchek SM, Rebbeck TR. Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst 2014;106:dju091.
- Friedenreich CM, Woolcott CG, McTiernan A, *et al*. Alberta physical activity and breast cancer prevention trial: sex hormone changes in a year-long exercise intervention among postmenopausal women. J Clin Oncol 2010;28:1458.
- Friedman GD, Ury HK. Initial screening for carcinogenicity of commonly used drugs. J Natl Cancer Inst 1980;65:723-33.
- Friedman LS, Ostermeyer EA, Szabo CI, *et al*. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nature Genet 1994;8:399-404.
- Friis S, Nielsen GL, Mellemkjaer L, McLaughlin JK, Thulstrup AM, Blot WJ, Lipworth L, Vilstrup H, Olsen JH. Cancer risk in persons receiving prescriptions for paracetamol: a Danish cohort study. Int J Cancer 2002;97:96-101.
- Friis S, Sørensen HT, McLaughlin JK, Johnsen SP, Blot WJ, Olsen JH. A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin. Br J Cancer 2003;88:684-8.
- Friis S, Thomassen L, Sørensen HT, Tjønneland A, Overvad K, Cronin-Fenton DP, Vogel U, McLaughlin JK, Blot WJ, Olsen JH. Nonsteroidal antiinflammatory drug use and breast cancer risk: a Danish cohort study. Eur J Cancer Prev 2008;17:88-96.

- Fulton AM, Ma X, Kundu N. Targeting prostaglandin E EP receptors to inhibit metastasis. Cancer Res 2006;66:9794–7.
- Funkhouser EM, Sharp GB. Aspirin and reduced risk of esophageal carcinoma. Cancer 1995;76:1116–9.
- Gabay C. Interleukin-6 and chronic inflammation. Arthritis Res Ther 2006;8:S3.
- Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Shcairer C, Mulvihil JJ. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 1989;81:1879-86.
- Gallicchio L, McSorley MA, Newschaffer CJ, Thuita LW, Huang HY, Hoffman SC, Helzlsouer KJ. Nonsteroidal antiinflammatory drugs, cyclooxygenase polymorphisms, and the risk of developing breast carcinoma among women with benign breast disease. Cancer 2006;106:1443-52.
- Gallicchio L, Visvanathan K, Burke A, Hoffman SC, Helzlsouer KJ. Nonsteroidal antiinflammatory drugs and the risk of developing breast cancer in a population-based prospective cohort study in Washington County, MD. Int J Cancer 2007;121:211-5.
- Gao X, Fisher SG, Emami B. Risk of second primary cancer in the contralateral breast in women treated for early-stage breast cancer: a population-based study. Int J Radiat Oncol Biol Phys 2003;56:1038–45.
- García Rodríguez LA, González-Pérez A. Risk of breast cancer among users of aspirin and other antiinflammatory drugs. Br J Cancer 2004;91:525-9.
- García-Esquinas E, Guinó E, Castaño-Vinyals G, Pérez-Gómez B, Llorca J, Altzibar JM, Peiró-Pérez R, Martín V, Moreno-Iribas C, Tardón A, Caballero FJ, Puig-Vives M, Guevara M, Villa TF, Salas D, Amiano P, Dierssen-Sotos T, Pastor-Barriuso R, Sala M, Kogevinas M, Aragonés N, Moreno V, Pollán M. Association of diabetes and diabetes treatment with incidence of breast cancer.Acta Diabetol 2015 [Epub ahead of print].
- Gargallo CJ, Lanas A. Is NSAIDs-related gastrointestinal damage preventable? J Dig Dis 2013;14:55-61.
- Garidou A, Tzonou A, Lipworth L, Signorello LB, Kalapothaki V, Trichopoulos D. Life-style factors and medical conditions in relation to esophageal cancer by histologic type in a low-risk population. Int J Cancer 1996;68:295–9.
- Gaudet MM, Kirchhoff T, Green T, Vijai J, Korn JM, Guiducci C, Segrè AV, McGee K, McGuffog L, Kartsonaki C, Morrison J, Healey S, Sinilnikova OM, Stoppa-Lyonnet D, Mazoyer S, Gauthier-Villars M, Sobol H, Longy M, Frenay M, GEMO Study Collaborators, Hogervorst FB, Rookus MA, Collée JM, Hoogerbrugge N, van Roozendaal KE; HEBON Study Collaborators, Piedmonte M, Rubinstein W, Nerenstone S, Van Le L, Blank SV, Caldés T, de la Hoya M, Nevanlinna H, Aittomäki K, Lazaro C, Blanco I, Arason A, Johannsson OT, Barkardottir RB, Devilee P, Olopade OI, Neuhausen SL, Wang X, Fredericksen ZS, Peterlongo P, Manoukian S, Barile M, Viel A, Radice P, Phelan CM, Narod S, Rennert G, Lejbkowicz F, Flugelman A, Andrulis IL, Glendon G, Ozcelik H; OCGN, Toland AE, Montagna M, D'Andrea E, Friedman E, Laitman Y, Borg A, Beattie M, Ramus SJ, Domchek SM, Nathanson KL, Rebbeck T, Spurdle AB, Chen X, Holland H; kConFab, John EM, Hopper JL, Buys SS, Daly MB, Southey MC, Terry MB, Tung N, Overeem Hansen TV, Nielsen FC, Greene MH, Mai PL, Osorio A, Durán M, Andres R, Benítez J, Weitzel JN, Garber J, Hamann U;

EMBRACE, Peock S, Cook M, Oliver C, Frost D, Platte R, Evans DG, Lalloo F, Eeles R, Izatt L, Walker L, Eason J, Barwell J, Godwin AK, Schmutzler RK, Wappenschmidt B, Engert S, Arnold N, Gadzicki D, Dean M, Gold B, Klein RJ, Couch FJ, Chenevix-Trench G, Easton DF, Daly MJ, Antoniou AC, Altshuler DM, Offit K. Common genetic variants and modification of penetrance of BRCA2-associated breast cancer. PLoS Genet 2010;6:e1001183.

- Gaudet MM, Press MF, Haile RW, Lynch CF, Glaser SL, Schildkraut J, Gammon MD, Douglas Thompson W, Bernstein JL. Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger. Breast Cancer Res Treat 2011;130:587-97.
- Genome Bioinformatics Group at Center for Biomolecular Science and Engineering UCSC genome bioinformatics. http://genome.cse.ucsc.edu/cgibin/hgGene?hgg_gene=uc002ico.1&hgg_prot=P38398&hgg_chrom=chr17&hgg_start=38449839&hgg_end=3853657&hgg_type=knownGene&db=hg18&hgsid=100101404. Accessed July 16, 2013.
- Ghoussaini M, Fletcher O, Michailidou K, Turnbull C, Schmidt MK, Dicks E, Dennis J, Wang Q, Humphreys MK, Luccarini C, Baynes C, Conroy D, Maranian M, Ahmed S, Driver K, Johnson N, Orr N, dos Santos Silva I, Waisfisz Q, Meijers-Heijboer H, Uitterlinden AG, Rivadeneira F, Netherlands Collaborative Group on Hereditary Breast and Ovarian Cancer (HEBON), Hall P, Czene K, Irwanto A, Liu J, Nevanlinna H, Aittomäki K, Blomqvist C, Meindl A, Schmutzler RK, MüllRE-Myhsok B, Lichtner P, Chang-Claude J, Hein R, Nickels S, Flesch-Janys D, Tsimiklis H, Makalic E, Schmidt D, Bui M, Hopper JL, Apicella C, Park DJ, Southey M, Hunter DJ, Chanock SJ, Broeks A, Verhoef S, Hogervorst FB, Fasching PA, Lux MP, Beckmann MW, Ekici AB, Sawyer E, Tomlinson I, Kerin M, Marme F, Schneeweiss A, Sohn C, Burwinkel B, Guénel P, Truong T, Cordina-Duverger E, Menegaux F, Bojesen SE, Nordestgaard BG, Nielsen SF, Flyger H, Milne RL, Alonso MR, González-Neira A, Benítez J, Anton-Culver H, Ziogas A, Bernstein L, Dur CC, Brenner H, Müller H, Arndt V, Stegmaier C, Familial Breast Cancer Study (FBCS), Justenhoven C, Brauch H, Brüning T, Gene Environment Interaction of Breast Cancer in Germany (GENICA) Network, Wang-Gohrke S, Eilber U, Dörk T, Schürmann P, Bremer M, Hillemanns P, Bogdanova NV, Antonenkova NN, Rogov YI, Karstens JH, Bermisheva M, Prokofieva D, Khusnutdinova E, Lindblom A, Margolin S, Mannermaa A, Kataja V, Kosma VM, Hartikainen JM, Lambrechts D, Yesilyurt BT, Floris G, Leunen K, Manoukian S, Bonanni B, Fortuzzi S, Peterlongo P, Couch FJ, Wang X, Stevens K, Lee A, Giles GG, Baglietto L, Severi G, McLean C, Alnaes GG, Kristensen V, Børrensen-Dale AL, John EM, Miron A, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Kauppila S, Andrulis IL, Glendon G, Mulligan AM, Devilee P, van Asperen CJ, Tollenaar RA, Seynaeve C, Figueroa JD, Garcia-Closas M, Brinton L, Lissowska J, Hooning MJ, Hollestelle A, Oldenburg RA, van den Ouweland AM, Cox A, Reed MW, Shah M, Jakubowska A, Lubinski J, Jaworska K, Durda K, Jones M, Schoemaker M, Ashworth A, Swerdlow A, Beesley J, Chen X, kConFab Investigators, Australian Ovarian Cancer Study Group, Muir KR, Lophatananon A, Rattanamongkongul S, Chaiwerawattana A, Kang D, Yoo KY, Noh DY, Shen CY, Yu JC, Wu PE, Hsiung CN, Perkins A, Swann R, Velentzis L, Eccles DM, Tapper WJ, Gerty SM, Graham NJ, Ponder BA, Chenevix-Trench G, Pharoah PD, Lathrop M, Dunning AM, Rahman N, Peto J, Easton DF. Genome-wide association analysis identifies three new breast cancer susceptibility loci. Nat Genet 2012;44:312-8.
- Gierach GL, Lacey JV Jr, Schatzkin A, Leitzmann MF, Richesson D, Hollenbeck AR, Brinton LA. Nonsteroidal antiinflammatory drugs and breast cancer risk in the National Institutes of Health-AARP Diet and Health Study. Breast Cancer Res 2008;10:R38.

- Gierach GL, Yang XR, Figueroa JD, Sherman ME. Emerging Concepts in Breast Cancer Risk Prediction. Current Obstetrics and Gynecology Reports 2013;2:43-52.
- Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, Dinan M, McBroom AJ, Hasselblad V, Sanders GD, Myers ER. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review.Cancer Epidemiol Biomarkers Prev 2013;22:1931-43.
- Gill JK, Maskarinec G, Wilkens LR, Pike MC, Henderson BE, Kolonel LN. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. Am J Epidemiol 2007;166:1150-8.
- Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC, Speizer FE. Aspirin and the risk of colorectal cancer in women. N Engl J Med 1995;333:609–14.
- Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206-23.
- González-Pérez A, García Rodríguez LA, López-Ridaura R. Effects of non-steroidal antiinflammatory drugs on cancer sites other than the colon and rectum: a meta-analysis. BMC Cancer 2003;3:28.
- Goss, PE, Ingle, JN, Ales-Martinez, JE *et al*. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med 2011;364:2381–91.
- Goya L, Maiyar AC, Ge Y, Firestone GL. Glucocorticoids induce a G1/G0 cell cycle arrest of Con8 rat mammary tumor cells that is synchronously reversed by steroid withdrawal or addition of transforming growth factor-alpha. Mol Endocrinol 1993;7:1121–32.
- Grabrick DM, Hartmann LC, Cerhan JR, *et al*. Risk of breast cancer with oral contraceptive use in women with a family history of breast cancer. JAMA 2000;284:1791.
- Grosch S, Maier TJ, Schiffmann S, Geisslinger G. Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors. J Natl Cancer Inst 2006;98:736–47.
- Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest 2006;116:4–15.
- Guibout C, Adjadj E, Rubino C, *et al*. Malignant breast tumors after radiotherapy for a first cancer during childhood. J Clin Oncol 2005;23:197.
- Gunnarsson C, Jansson A, Holmlund B, Ferraud L, Nordenskjöld B, Rutqvist LE, Skoog L, Stål O. Expression of COX-2 and steroid converting enzymes in breast cancer. Oncol Rep 2006;16: 219-24.
- Gunter MJ, Hoover DR, Yu H, *et al*. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2009;101:48.
- Half E, Tang XM, Gwyn K, Sahin A, Wathen K, Sinicrope FA. Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. Cancer Res 2002;62:1676–81.
- Hall JM, Lee MK, Newman B, *et al*. . Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990;250:1684-9.

- Hamid R, Singh J, Reddy BS, Cohen LA. Inhibition by dietary menhaden oil of cyclooxygenase-1 and -2 in N-nitrosomethylurea-induced rat mammary tumors. Int J Oncol 1999;14:523–28.
- Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. N Engl J Med 2003;348:2313-22.
- Hankinson S, Tamimi R, Hunter D. Breast Cancer. En: Adami HO, Hunter D, Trichopoulos D, MacMahon B. Textbook of Cancer Epidemiology. Oxford: Oxford University Press; 2008. p. 403-45.
- Hankinson SE, Colditz GA, Manson JE, *et al*. . A prospective study of oral contraceptive use and risk of breast cancer (Nurses' Health Study, United States). Cancer Causes Control 1997;8:65.
- Hannaford PC, Selvaraj S, Elliott AM, *et al*. Cancer risk among users of oral contraceptives: cohort data from the Royal College of General Practitioner's oral contraception study. BMJ 2007;335:651.
- Harris R, Namboodiri K, Farrar W. Epidemiologic-study of nonsteroidal antiinflammatory drugs and breast-cancer. Oncol Rep 1995;2:591-2.
- Harris RE, Alshafie GA, Abou-Issa H, Seibert K. Chemo- prevention of breast cancer in rats by celecoxib, a cyclooxy- genase 2 inhibitor. Cancer Res 2000; 60: 2101-2103 [PMID: 10786667]
- Harris RE, Alshafie GA, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000;20:2101–3.
- Harris RE, Beebe-Donk J, Alshafie GA. Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer 2006;6:27.
- Harris RE, Beebe-Donk J, Doss H, Burr Doss D. Aspirin, ibuprofen, and other non-steroidal antiinflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade. Oncol Rep 2005;13:559-83.
- Harris RE, Casto BC, Harris ZM. Cyclooxygenase-2 and the inflammogenesis of breast cancer. World J Clin Oncol 2014;5:677-92.
- Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, McTiernan A; Women's Health Initiative. Breast cancer and nonsteroidal antiinflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res 2003;63:6096-101.
- Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, McTiernan A. Breast cancer and nonsteroidal antiinflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res 2003;63:6096–101.
- Harris RE, Kasbari S, Farrar WB. Prospective study of nonsteroidal antiinflammatory drugs and breast cancer. Oncol Rep 1999;6:71-3.
- Harris RE, Namboodiri K, Stellman SD, Wynder EL. Breast cancer and NSAID use: heterogeneity of effect in a case-control study. Prev Med 1995;24(2):119-20.
- Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. Epidemiology 1996;7:203-5.

- Harris RE, Robertson FM, Abou-Issa HM, Farrar WB, Brueggemeier R. Genetic induction and upregulation of cyclo- oxygenase (COX) and aromatase (CYP19): an extension of the dietary fat hypothesis of breast cancer. Med Hypotheses 1999;52:291-2.
- Harris RE. Cyclooxygenase-2 (cox-2) and the inflammogenesis of cancer. Subcell Biochem 2007;42:93-126.
- Harris RE. Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. Inflammopharmacology 2009;17:55-67.
- Harris RE. Cyclooxygenase-2 blockade in cancer prevention and therapy: widening the scope of impact. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002:341-65.
- Hartmann LC, Lingle W, Frost MH, Shaun D, Maloney RA, Vierkant V, Pankratz S, Tisty T, Degnim AC, Visscher DW. COX-2 expression in atypia: correlation with breast cancer risk. Proc Amer Assoc Cancer Res 97th Annual Meeting, 2006: Abstract No. 2353
- He TC. Association of COX-2 and PPARs in carcinogenesis and chemoprevention. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002: 225-44 .
- Healy LA, Ryan AM, Carroll P, *et al*. Metabolic syndrome, central obesity and insulin resistance are associated with adverse pathological features in postmenopausal breast cancer. Clin Oncol (R Coll Radiol) 2010;22:281–8.
- Hecht SS. Tobacco smoke carcinogens and breast cancer. Environ Mol Mutagen 2002;39:119-26.
- Hedenfalk I, Duggan D, Chen Y, *et al*. Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001;344:539-48.
- Heikkinen T, Kärkkäinen H, Aaltonen K, Milne RL, Heikkilä P, Aittomäki K, Blomqvist C, Nevanlinna H. The breast cancer susceptibility mutation PALB2 1592delT is associated with an aggressive tumor phenotype. Clin Cancer Res. 2009;15:3214-22.
- Heiss G, Wallace R, Anderson GL, Aragaki A, Beresford SA, Brzyski R, Chlebowski RT, Gass M, LaCroix A, Manson JE, Prentice RL, Rossouw J, Stefanick ML; WHI Investigators. Health risks and benefits 3 years after stopping randomized treatment with estrogen and progestin. JAMA 2008;299:1036-45.
- Hemachandra LP, Patel H, Chandrasena RE, Choi J, Piyankarage SC, Wang S, Wang Y, Thayer EN, Scism RA, Michalsen BT, Xiong R, Siklos MI, Bolton JL, Thatcher GR. SERMs attenuate estrogen-induced malignant transformation of human mammary epithelial cells by upregulating detoxification of oxidative metabolites. Cancer Prev Res 2014;7:505-15.
- Henderson BR. Nuclear-cytoplasmic shuttling of APC regu- lates beta-catenin subcellular localization and turnover. Nat Cell Biol 2000;2:653-60.
- Henderson TO, Amsterdam A, Bhatia S, *et al*. Systematic review: surveillance for breast cancer in women treated with chest radiation for childhood, adolescent, or young adult cancer. Ann Intern Med 2010;152:444.
- Herschkowitz, J., Simin, K., Weigman, V., Mikaelian, I., Usary, J.,Hu, Z., Rasmussen, K., Jones, L.,Assefnia, S., Chandrasekharan, S., Backlund, M., Yin, Y., Khramtsov, A., Bastein, R.,

Quackenbush, J., Glazer, R., Brown, P., Green, J., Kopelovich, L., Furth, P., Palazzo, J., Olopade, O., Bernard, P., Churchill, G., Van Dyke, T., Perou, C., 2007. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 8, R76.

- Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta 1996;1299:125–40.
- Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
- Hindorff LA, MacArthur J (European Bioinformatics Institute), Morales J (European Bioinformatics Institute), Junkins HA, Hall PN, Klemm AK, and Manolio TA. A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/gwastudies. Accessed [12/22/14]
- Hirose H, Ishii H, Mimori K, Ohta D, Ohkuma M, Tsujii H, Saito T, Sekimoto M, Doki Y, Mori M. Notch pathway as candidate therapeutic target in Her2/Neu/ErbB2 receptor-negative breast tumors. Oncol Rep 2010;23:35-43.
- Hodgson SV, Morrison PJ, Irving M. Breast cancer genetics: unsolved questions and open perspectives in an expanding clinical practice. Am J Med Genet 2004;129:56-64.
- Hodis HN, Mack WJ. Hormone replacement therapy and the association with coronary heart disease and overall mortality: clinical application of the timing hypothesis. J Steroid Biochem Mol Biol 2014;142:68-75.
- Hopper JL, Hayes VM, Spurdle AB, *et al*. A protein-truncating mutation in CYP17A1 in three sisters with early-onset breast cancer. Hum Mutat 2005;26:298-302.
- Howe LR, Chang SH, Tolle KC, Dillon R, Young LJT, Cardiff RD, Newman RA, Yang P, Thaler HT, Muller WJ, Hudis C, Brown AMC, Hla T, Subbaramaiah K, Dannenberg AJ. HER2/neu-induced mammary tumorigenesis and angiogenesis are reduced in cyclooxygenase-2 knockout mice. Cancer Research 2005;65:10113–9.
- Howe LR, Crawford HC, Subbaramaiah K, Hassell JA, Dannenberg AJ, Brown AMC. PEA3 is upregulated in response to Wnt1 and activates the expression of cyclooxygenase-2. J Biol Chem 2001;276:20108–5.
- Howe LR, Lippman SM. Modulation of breast cancer risk by nonsteroidal antiinflammatory drugs. J Natl Cancer Inst 2008;100:1420-3.
- Howe LR, Subbaramaiah K, Brown AM, Dannenberg AJ. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. Endocr Relat Cancer 2001;8:97-114.
- Howe LR, Subbaramaiah K, Patel J, Masferrer JL, Deora A, Hudis C, Thaler HT, Muller WJ, Du B, Brown AMC, *et al*. . Celecoxib, a selective cyclooxygenase-2 inhibitor, protects against human epidermal growth factor receptor 2 (HER-2)/neu-induced breast cancer. Cancer Res 2002;62:5405–7.
- Howe LR. Cyclooxygenase-2 and breast cancer. In: Yao AP, editor. Trends in Breast Cancer Research. Vol. 9. New York: Nova; 2005. pp. 1–38.
- Howe LR. Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. Breast Cancer Res 2007;9:210.

- Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2011, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2011/, based on November 2013 SEER data submission, posted to the SEER web site, April 2014.
- Hu P, Huang Q, Li Z, Wu X, Ouyang Q, Chen J, Cao Y.Silencing MAP3K1 expression through RNA interference enhances paclitaxel-induced cell cycle arrest in human breast cancer cells. Mol Biol Rep 2014;41:19-24.
- Hunter DJ, Kraft P, Jacobs KB, *et al*. "A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer". Nature Genet 2007;39:870–4.
- Hurh YJ, Chen ZH, Na HK, Han SY, Surh YJ. 2-Hydroxyestradiol induces oxidative DNA damage and apoptosis in human mammary epithelial cells. J Toxicol Environ Health A 2004;67:1939-53.
- Hursting SD, Smith SM, Lashinger LM, Harvey AE, Perkins SN. Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. Carcinogenesis 2010;31:83–9.
- Hussain, Shobbir; Wilson James B, Medhurst Annette L, Hejna James, Witt Emily, Ananth Sahana, Davies Adelina, Masson Jean-Yves, Moses Robb, West Stephen C, de Winter Johan P, Ashworth Alan, Jones Nigel J, Mathew Christopher G. Direct interaction of FANCD2 with BRCA2 in DNA damage response pathways. Hum Mol Genet 2004 13:1241–8.
- Hwang D, Scollard D, Byrne J , Levine E . Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst 1998;90:455–60.
- IARC (2010). Painting, firefighting, and shiftwork. IARC Monogr Eval Carcinog Risks Hum, 98:9-764.
- Iniesta MD, Gorin MA, Chien LC, Thomas SM, Milliron KJ, Douglas JA, Merajver SD. Absence of CHEK2*1100delC mutation in families with hereditary breast cancer in North America. Cancer Genet Cytogenet 2010;202:136-40.
- Irwin ML, Aiello EJ, McTiernan A, *et al*. Physical activity, body mass index, and mammographic density in postmenopausal breast cancer survivors. J Clin Oncol 2007; 25:1061.
- Isaacs C, Fletcher SW, Peshkin BN. Genetic testing for hereditary breast and ovarian cancer syndrome. En: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA, 2012.
- Jacobs EJ, Thun MJ, Bain EB, Rodriguez C, Henley SJ, Calle EE. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. J Natl Cancer Inst 2007;99:608-15.
- Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, Patel AV, Flanders WD, Calle EE. Aspirin and other nonsteroidal antiinflammatory drugs and breast cancer incidence in a large US cohort. Cancer Epidemiol Biomarkers Prev 2005;14261-4.
- Jang BC, Hla T. Regulation of expression and potential carcinogenic role of cylcooxygenase-2. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002: 171-84.
- Jensen RB, Ozes A, Kim T, Estep A, Kowalczykowski SC. BRCA2 is epistatic to the RAD51 paralogs in response to DNA damage. DNA Repair 2013;12:306-11.

- Jernström H, Lubinski J, Lynch HT, *et al*. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2004;96:1094.
- Jin ZC, Zhou XH, He J. Statistical methods for dealing with publication bias in meta-analysis. Stat Med 2015;34:343-60.
- Joarder F, Abouissa H, Robertson F, Parrett M, Alshafie G, Harris R. Growth arrest of DMBA-induced mammary car- cinogenesis with ibuprofen treatment in female Sprague- Dawley rats. Oncol Rep 1997;4:1271-73.
- John EM, Kelsey JL. Radiation and other environmental exposures and breast cancer. Epidemiol Rev 1993;15:157.
- Johnson KC, Miller AB, Collishaw NE, Palmer JR, Hammond SK, Salmon AG, Cantor KP, Miller MD, Boyd NF, Millar J, Turcotte F.Active smoking and secondhand smoke increase breast cancer risk: the report of the Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk (2009). Tob Control 2011;20:e2.
- Johnson TW, Anderson KE, Lazovich D, Folsom AR. Association of aspirin and nonsteroidal antiinflammatory drug use with breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1586-91.
- Jonsson F, Yin L, Lundholm C, Smedby KE, Czene K, Pawitan Y. Low-dose aspirin use and cancer characteristics: a population-based cohort study. Br J Cancer 2013;109:1921-5.
- Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, Dossus L, Lukanova A, Bingham S, Khaw KT, Allen NE, Bueno-de-Mesquita HB, van Gils CH, Grobbee D, Boeing H, Lahmann PH, Nagel G, Chang-Claude J, Clavel-Chapelon F, Fournier A, Thiébaut A, González CA, Quirós JR, Tormo MJ, Ardanaz E, Amiano P, Krogh V, Palli D, Panico S, Tumino R, Vineis P, Trichopoulou A, Kalapothaki V, Trichopoulos D, Ferrari P, Norat T, Saracci R, Riboli E. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. Endocr Relat Cancer 2005; 12:1071-82.
- Kabat GC, Anderson ML, Heo M, Hosgood HD 3rd, Kamensky V, Bea JW, Hou L, Lane DS, Wactawski-Wende J, Manson JE, Rohan TE. Adult stature and risk of cancer at different anatomic sites in a cohort of postmenopausal women. Cancer Epidemiol Biomarkers Prev 2013;22:1353-63.
- Kabat GC, Kim MY, Hollenbeck AR, Rohan TE. Attained height, sex, and risk of cancer at different anatomic sites in the NIH-AARP diet and health study. Cancer Causes Control 2014;25:1697-706.
- Kaklamani VG, Baddi L, Liu J, Rosman D, Phukan S, Bradley C, Hegarty C, McDaniel B, Rademaker A, Oddoux C, Ostrer H, Michel LS, Huang H, Chen Y, Ahsan H, Offit K, Pasche B. Combined genetic assessment of transforming growth factor-beta signaling pathway variants may predict breast cancer risk. Cancer Res 2005;65:3454-61.
- Kang JH, Song KH, Jeong KC, Kim S, Choi C, Lee CH, Oh SH. Involvement of Cox-2 in the metastatic potential of che- motherapy-resistant breast cancer cells. BMC Cancer 2011;11:334.
- Karmali RA, Marsh J, Fuchs C. Effect of omega-3 fatty acids on growth of a rat mammary tumor. J Natl Cancer Inst 1984;73:457-61.

- Karmali RA, Marsh J. Antitumor activity in a rat mammary adenocarcinoma: the effect of cyclooxygenase inhibitors and immunization against prostaglandin E2. Prostaglandins Leu- kot Med 1985;20:283-6.
- Karmali RA. Dietary fatty acids, COX-2 blockade, and carci- nogenesis. In: RE Harris, ed. COX-2 Blockade in Cancer Pre- vention and Therapy. Humana Press, Totowa, NJ, 2002:3-12
- Karppinen SM, Heikkinen K, Rapakko K, Winqvist R. Mutation screening of the BARD1 gene: evidence for involvement of the Cys557Ser allele in hereditary susceptibility to breast cancer. J Med Genet 2004;41:114.
- Keller BM, Chen J, Conant EF, Kontos D. Breast density and parenchymal texture measures as potential risk factors for Estrogen-Receptor positive breast cancer. Proceedings of SPIE 2014;9035:90351D.
- Kelly LM, Hill AD, Kennedy S, Connolly EM, Ramanath R, Teh S, Dijkstra B, Purcell R, McDermott EW, O'Higgins N. Lack of prognostic effect of Cox-2 expression in primary breast cancer on short-term follow-up. Eur J Surg Oncol 2003;29:707–10.
- Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993;15:36-47.
- Kelsey JL. A review of the epidemiology of human breast cancer. Epidemiol Rev 1979;1:74-109.
- Kenney LB, Yasui Y, Inskip PD, *et al*. Breast cancer after childhood cancer: a report from the Childhood Cancer Survivor Study. Ann Intern Med 2004;141:590.
- Kerlikowske K, Ichikawa L, Miglioretti DL, *et al*. Longitudinal measurement of clinical mammographic breast density to improve estimation of breast cancer risk. J Natl Cancer Inst 2007;99:386.
- Kerlikowske K, Shepherd J, Creasman J, *et al*. Are breast density and bone mineral density independent risk factors for breast cancer? J Natl Cancer Inst 2005; 97:368.
- Key T, Appleby P, Barnes I, *et al*. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002; 94:606.
- Khan SA, Rogers MA, Khurana KK, Meguid MM, Numann PJ. Estrogen receptor expression in benign breast epithelium and breast cancer risk. J Natl Cancer Inst 1998;90:37-42.
- Khuder SA, Mutgi AB. Breast cancer and NSAID use: a meta-analysis. Br J Cancer 2001;84:1188-92.
- Kinoshita T, Takahashi Y, Sakashita T, Inoue H, Tanabe T, Yoshimoto T. Growth stimulation and induction of epider- mal growth factor receptor by overexpression of cyclooxy- genases 1 and 2 in human colon carcinoma cells. Biochim Biophys Acta 1999;1438:120-30.
- Kirkpatrick K, Ogunkolade W, Elkak A, Bustin S, Jenkins P, Ghilchik M, Mokbel K. The mRNA expression of cyclooxygenase- 2 (COX-2) and vascular endothelial growth factor (VEGF) in human breast cancer. Curr Med Res Opin 2002;18:237-41.
- Kirsh VA, Kreiger N, Cotterchio M, Sloan M, Theis B. Nonsteroidal antiinflammatory drug use and breast cancer risk: subgroup findings. Am J Epidemiol 2007;166:709-16.

- Kitchen AJ, Trivedi P, Ng D, Mokbel K. Is there a link between breast cancer and abortion: a review of the literature. Int J Fertil Womens Med 2005;50:267-71.
- Koki AT, Leahy KM, Harmon JM, Masferrer JL. Cyclooxygenase-2 and cancer. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002: 185-203.
- Kopans DB. Basic physics and doubts about relationship between mammographically determined tissue density and breast cancer risk. Radiology 2008;246:348.
- Kort WJ, Weijma IM, Bijma AM, van Schalkwijk WP, Vergroesen AJ, Westbroek DL. Omega-3 fatty acids inhibiting the growth of a transplantable rat mammary adenocarcinoma. Journal of the National Cancer Institute 1987;79:593–9.
- Kotsopoulos J, Lubinski J, Salmena L, Lynch HT, Kim-Sing C, Foulkes WD, Ghadirian P, Neuhausen SL, Demsky R, Tung N, Ainsworth P, Senter L, Eisen A, Eng C, Singer C, Ginsburg O, Blum J, Huzarski T, Poll A, Sun P, Narod SA; Hereditary Breast Cancer Clinical Study Group. Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res 2012;14:R42.
- Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Bahadori B, Samonigg H. The L10P polymorphism of the transforming growth factor-beta 1 gene is not associated with breast cancer risk. Cancer Lett 2003;201:181-4.
- Krontiris TG, Devlin B, Karp DD, Robert NJ, Risch N. An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N Engl J Med 1993;329:517-23.
- Kubatka P, Ahlers I, Ahlersova E, Adamekova E, Luk P, Bojkova B, Markova M. Chemoprevention of mammary carcinogenesis in female rats by rofecoxib. Cancer Lett 2003;202:131–6.
- Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res 1988;48:4399–404.
- Labat ML. Possible retroviral etiology of human breast cancer. Biomed Pharmacother 1998;52:6-12.
- Lahmann PH, Hoffmann K, Allen N, *et al*. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer 2004; 111:762.
- Lanfranchi A. Normal breast physiology: the reasons hormonal contraceptives and induced abortion increase breast-cancer risk. Issues Law Med 2014;29:135-46.
- Lange CA, Sartorius CA, Abdel-Hafiz H, Spillman MA, Horwitz KB, Jacobsen BM. Progesterone receptor action: translating studies in breast cancer models to clinical insights. Adv Exp Med Biol 2008;630:94-111.
- Langman MJ, Cheng KK, Gilman EA, Lancashire RJ. Effect of antiinflammatory drugs on overall risk of common cancer: case-control study in general practice research database. BMJ. 2000;320:1642-6.
- Lanza-Jacoby S, Miller S, Flynn J, Gallatig K, Daskalakis C, Masferrer JL, Zweifel BS, Sembhi H, Russo IH. The cyclooxygenase-2 inhibitor, celecoxib, prevents the development of mammary tumors in Her-2/neu mice. Cancer Epidemiol Biomarkers Prev 2003;12:1486–91.

- Larkins TL, Nowell M, Singh S, Sanford GL. Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. BMC Cancer 2006;6: 181.
- Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol 2009;1:a001651.
- Lawson JS, Petridou E, Trichopoulos D. Possible permanent down regulation of oestrogen receptor expression during fetal life and breast cancer risk. Br J Cancer 1999;80:1678.
- Le Marchand L, Haiman CA, van den Berg D, Wilkens LR, Kolonel LN, Henderson BE. T29C polymorphism in the transforming growth factor beta1 gene and postmenopausal breast cancer risk: the Multiethnic Cohort Study. Cancer Epidemiol Biomarkers Prev 2004;13:412-5.
- Lee IM, Cook NR, Rexrode KM, Buring JE. Lifetime physical activity and risk of breast cancer. Br J Cancer 2001;85:962.
- Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 2002;404:201-4.
- Lee PS, Fang J, Jessop L, Myers T, Raj P, Hu N, Wang C, Taylor PR, Wang J, Khan J, Jasin M, Chanock SJ. RAD51B Activity and Cell Cycle Regulation in Response to DNA Damage in Breast Cancer Cell Lines. Breast Cancer 2014;8:135-44.
- Levitt RJ, Buckley J, Blouin MJ, Schaub B, Triche TJ, Pollak M. Growth inhibition of breast epithelial cells by celecoxib is associated with upregulation of insulin-like growth fac- tor binding protein-3 expression. Biochem Biophys Res Commun 2004;316:421-428.
- Li CI, Anderson BO, Daling JR, Moe RE. Trends in incidence rates of invasive lobular and ductal breast carcinoma. JAMA 2003;289:1421-4.
- Li CI, Malone KE, Porter PL, *et al*. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA 2003;289:3254.
- Li FP. Familial cancer syndromes and clusters. Curr Prob Cancer 1990;14:73-114.
- Li J, Humphreys K, Heikkinen T, Aittomäki K, Blomqvist C, Pharoah PD, Dunning AM, Ahmed S, Hooning MJ, Martens JW, van den Ouweland AM, Alfredsson L, Palotie A, Peltonen-Palotie L, Irwanto A, Low HQ, Teoh GH, Thalamuthu A, Easton DF, Nevanlinna H, Liu J, Czene K, Hall P. A combined analysis of genome-wide association studies in breast cancer. Breast Cancer Res Treat 2011;126:717-27.
- Li Z, Sergent F, Bolla M, Zhou Y, Gabelle-Fandin I. Prognostic factors of second primary contralateral breast cancer in early-stage breast cancer. Oncology Letters 2015;9:245-51.
- Liehr JG. Hormone-associated cancer: mechanistic similari- ties between human breast cancer and estrogen-induced kidney carcinogenesis in hamsters. Environ Health Perspect 1997;105:565-9.
- Light RJ, Pillemer DB. Summing up: The science of reviewing research. Harvard University Press.: Cambridge, MA, 1984.
- Lim SC. Role of COX-2, VEGF and cyclin D1 in mammary infiltrating duct carcinoma. Oncol Rep 2003;10:1241–9.

- Lindström S, Thompson DJ, Paterson AD, Li J, Gierach GL, Scott C, Stone J, Douglas JA, dos-Santos-Silva I, Fernandez-Navarro P, Verghase J, Smith P, Brown J, Luben R, Wareham NJ, Loos RJ, Heit JA, Pankratz VS, Norman A, Goode EL, Cunningham JM, deAndrade M, Vierkant RA, Czene K, Fasching PA, Baglietto L, Southey MC, Giles GG, Shah KP, Chan HP, Helvie MA, Beck AH, Knoblauch NW, Hazra A, Hunter DJ, Kraft P, Pollan M, Figueroa JD, Couch FJ, Hopper JL, Hall P, Easton DF, Boyd NF, Vachon CM, Tamimi RM. Genome-wide association study identifies multiple loci associated with both mammographic density and breast cancer risk. Nat Commun 2014;5:5303.
- Lippman ME, Krueger KA, Eckert S, *et al*. Indicators of lifetime estrogen exposure: effect on breast cancer incidence and interaction with raloxifene therapy in the multiple outcomes of raloxifene evaluation study participants. J Clin Oncol 2001;19:3111.
- Litton JK, Gonzalez-Angulo AM, Warneke CL, *et al*. Relationship between obesity and pathologic response to neoadjuvant chemotherapy among women with operable breast cancer. J Clin Oncol 2008;26:4072–7.
- Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T. Overexpression of cycloox- ygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001; 276: 18563-18569 [PMID: 11278747 DOI: 10.1074/jbc.M010787200]
- Lobaccaro JM, Lumbroso S, Belon C, *et al*. Androgen receptor gene mutation in male breast cancer. Hum Mol Genet 1993;2:1799-802.
- Long J, Cai Q, Sung H, Shi J, Zhang B, Choi JY, Wen W, Delahanty RJ, Lu W, Gao YT, Shen H, Park SK, Chen K, Shen CY, Ren Z, Haiman CA, Matsuo K, Kim MK, Khoo US, Iwasaki M, Zheng Y, Xiang YB, Gu K, Rothman N, Wang W, Hu Z, Liu Y, Yoo KY, Noh DY, Han BG, Lee MH, Zheng H, Zhang L, Wu PE, Shieh YL, Chan SY, Wang S, Xie X, Kim SW, Henderson BE, Le Marchand L, Ito H, Kasuga Y, Ahn SH, Kang HS, Chan KY, Iwata H, Tsugane S, Li C, Shu XO, Kang DH, Zheng W. Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. PLoS Genet 2012;8:e1002532.
- Long-term oral contraceptive use and the risk of breast cancer. The centers for Disease Control Cancer and Steroid Hormone Study. JAMA 1983;249:1591.
- Loveday C, Turnbull C, Ramsay E, *et al*. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet 2011;43:879–882.
- Luo J, Margolis KL, Wactawski-Wende J, Horn K, Messina C, Stefanick ML, Tindle HA, Tong E, Rohan TE. Association of active and passive smoking with risk of breast cancer among postmenopausal women: a prospective cohort study. BMJ 2011;342:d1016.
- Luo T, Yan HM, He P, Luo Y, Yang YF, Zheng H. Aspirin use and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 2012;131:581-7.
- Lupulescu, A. Enhancement of carcinogenesis by prostaglandins. Nature 1978;272:634-6.
- Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. Endocrinology 2003;144:2195-200.
- Ma H, Bernstein L, Pike MC, Ursin G. Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies. Breast Cancer Res 2006;8:R43.

- Ma H, Wang Y, Sullivan-Halley J, Weiss L, Marchbanks PA, Spirtas R, Ursin G, Burkman RT, Simon MS, Malone KE, Strom BL, McDonald JA, Press MF, Bernstein L. Use of four biomarkers to evaluate the risk of breast cancer subtypes in the women's contraceptive and reproductive experiences study. Cancer Res. 2010;70:575-87.
- MacPherson G, Healey CS, Teare MD, Balasubramanian SP, Reed MW, Pharoah PD, Ponder BA, Meuth M, Bhattacharyya NP, Cox A. Association of a common variant of the CASP8 gene with reduced risk of breast cancer. J Natl Cancer Inst 2004;96:1866-9.
- Magnusson C, Baron JA, Correia N, Bergström R, Adami HO, Persson I. Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy. Int J Cancer 1999;81:339-44.
- Maihöfner C, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G, Gooderham NJ. Expression of cyclooxygenase- 2 parallels expression of interleukin-1beta, interleukin-6 and NF-kappaB in human colorectal cancer. Carcinogenesis 2003;24:665-71.
- Mangiapane S, Blettner M, Schlattmann P. Aspirin use and breast cancer risk: a meta-analysis and meta-regression of observational studies from 2001 to 2005. Pharmacoepidemiol Drug Saf 2008;17:115-24.
- Manjer J, Johansson R, Berglund G, Janzon L, Kaaks R, Agren A, Lenner P. Postmenopausal breast cancer risk in relation to sex steroid hormones, prolactin and SHBG. Cancer Causes Control 2003 Sep; 14:599-607.
- Mann JR, Backlund MG, Buchanan FG, Daikoku T, Holla VR, Rosenberg DW, Dey SK, DuBois RN. Repression of prostaglandin dehydrogenase by epidermal growth factor and snail increases prostaglandin E2 and promotes cancer progression. Cancer Res 2006;66:6649–56.
- Marchbanks PA, McDonald JA, Wilson HG, Folger SG, Mandel MG, Daling JR, Bernstein L, Malone KE, Ursin G, Strom BL, Norman SA, Wingo PA, Burkman RT, Berlin JA, Simon MS, Spirtas R, Weiss LK. Oral contraceptives and the risk of breast cancer. N Engl J Med 2002;346:2025-32.
- Marnett LJ, Basu AK, O'Hara SM, Weller PE, Rahman AFMM, Oliver JP. Reaction of malondialdehyde with gua- nine nucleosides: formation of adducts containing oxadraza-bicyclononene residues in the base-pairing region. J Am Chem Soc 1986;108:1348-50.
- Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res 1999;424:83-95.
- Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A, Ross RK. Nonsteroidal antiinflammatory drug use and breast cancer risk by stage and hormone receptor status. J Natl Cancer Inst 2005;97:805-12.
- Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ, Seibert K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60:1306-11.
- Maskarinec G, Beckford F, Morimoto Y, Franke AA, Stanczyk FZ. Association of estrogen measurements in serum and urine of premenopausal women. Biomark Med. 2015;9:417-24.
- Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol 2010;4:174-91.

- McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006;15:1159.
- McCormick, DL, Wilson AM. Combination chemoprevention of rat mammary carcinogenesis by indomethacin and butylated hydroxytoluene. Cancer Res 1986;46:3907-11
- McDonnell DP, Chang CY and Norris JD Capitalizing on the complexities of estrogen receptor pharmacology in the quest for the perfect SERM Ann NY Acad Sci 2001;949:16-35.
- McDonnell DP. Selective estrogen receptor modulators (SERMs): A first step in the development of perfect hormone replacement therapy regimen Soc Gyn Invest 2000;7:S10-S15.
- McGettigan P, Henry D. Cardiovascular risk with non- steroidal antiinflammatory drugs: systematic review of population-based controlled observational studies. PLoS Med 2011;8: e1001098.
- McKusick V. Cancer of the breast and female reproductive tract. In: Rimoin D, Connor M, Pyeritz R, eds. Principles and Practice of Medical Genetics. New York: Churchill Livingstone, 1997: 2093-121.
- McPherson K, Steel CM, Dixon JM ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ 2000;321:624-8.
- McTiernan A, Tworoger SS, Ulrich CM, *et al*. Effect of exercise on serum estrogens in postmenopausal women: a 12-month randomized clinical trial. Cancer Res 2004;64:2923.
- Megdal SP, Kroenke CH, Laden F, Pukkala E, Schernhammer ES. Night work and breast cancer risk: a systematic review and metaanalysis. Eur J Cancer 2005;41:2023-32
- Mehrotra S, Morimiya A, Agarwal B, Konger R, Badve S. Microsomal prostaglandin E2 synthase-1 in breast cancer: a potential target for therapy. J Pathol 2006;208:356-63.
- Meier CR, Schmitz S, Jick H. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. Pharmacotherapy 2002;22:303-9.
- Meilahn EN, De Stavola B, Allen DS, Fentiman I, Bradlow HL, Sepkovic DW, Kuller LH. Do urinary oestrogen metabolites predict breast cancer? Guernsey III cohort follow-up. Br J Cancer 1998;78:1250–5.
- Meindl A, Hellebrand H, Wiek C, *et al*. . Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet 2010;42:410-4.
- Melana SM, Nepomnaschy I, Hasa J, Djougarian A, Djougarian A, Holland JF, Pogo BG. Detection of human mammary tumor virus proteins in human breast cancer cells. J Virol Methods 2010;163:157-61.
- Melbye M, Wohlfahrt J, Olsen JH, *et al*. . Induced abortion and the risk of breast cancer. N Engl J Med 1997;336:81.
- Mellemkjaer L, Linet MS, Gridley G, Frisch M, Moller H, Olsen JH: Rheumatoid arthritis and cancer risk. Eur J Cancer 1996;32A:1753-57.
- Menashe I, Maeder D, Garcia-Closas M, Figueroa JD, Bhattacharjee S, Rotunno M, Kraft P, Hunter DJ, Chanock SJ, Rosenberg PS, Chatterjee N. Pathway analysis of breast cancer genome-

wide association study highlights three pathways and one canonical signaling cascade. Cancer Res 2010;70:4453-9.

- Mestre JR, Subbaramaiah K, Sacks PG, Schantz SP, Tanabe T, Inoue H, Dannenberg AJ. Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells. Cancer Res 1997;57:2890-5.
- Michailidou K, Beesley J, Lindstrom S, Canisius S, Dennis J, Lush MJ, Maranian MJ, Bolla MK, Wang Q, Shah M, Perkins BJ, Czene K, Eriksson M, Darabi H, Brand JS, Bojesen SE, Nordestgaard BG, Flyger H, Nielsen SF, Rahman N, Turnbull C; BOCS, Fletcher O, Peto J, Gibson L, Dos-Santos-Silva I, Chang-Claude J, Flesch-Janys D, Rudolph A, Eilber U, Behrens S, Nevanlinna H, Muranen TA, Aittomäki K, Blomqvist C, Khan S, Aaltonen K, Ahsan H, Kibriya MG, Whittemore AS, John EM, Malone KE, Gammon MD, Santella RM, Ursin G, Makalic E, Schmidt DF, Casey G, Hunter DJ, Gapstur SM, Gaudet MM, Diver WR, Haiman CA, Schumacher F, Henderson BE, Le Marchand L, Berg CD, Chanock SJ, Figueroa J, Hoover RN, Lambrechts D, Neven P, Wildiers H, van Limbergen E, Schmidt MK, Broeks A, Verhoef S, Cornelissen S, Couch FJ, Olson JE, Hallberg E, Vachon C, Waisfisz Q, Meijers-Heijboer H, Adank MA, van der Luijt RB, Li J, Liu J, Humphreys K, Kang D, Choi JY, Park SK, Yoo KY, Matsuo K, Ito H, Iwata H, Tajima K, Guénel P, Truong T, Mulot C, Sanchez M, Burwinkel B, Marme F, Surowy H, Sohn C, Wu AH, Tseng CC, Van Den Berg D, Stram DO, González-Neira A, Benitez J, Zamora MP, Perez JI, Shu XO, Lu W, Gao YT, Cai H, Cox A, Cross SS, Reed MW, Andrulis IL, Knight JA, Glendon G, Mulligan AM, Sawyer EJ, Tomlinson I, Kerin MJ, Miller N; kConFab Investigators; AOCS Group, Lindblom A, Margolin S, Teo SH, Yip CH, Taib NA, Tan GH, Hooning MJ, Hollestelle A, Martens JW, Collée JM, Blot W, Signorello LB, Cai Q, Hopper JL, Southey MC, Tsimiklis H, Apicella C, Shen CY, Hsiung CN, Wu PE, Hou MF, Kristensen VN, Nord S, Alnaes GI; NBCS, Giles GG, Milne RL, McLean C, Canzian F, Trichopoulos D, Peeters P, Lund E, Sund M, Khaw KT, Gunter MJ, Palli D, Mortensen LM, Dossus L, Huerta JM, Meindl A, Schmutzler RK, Sutter C, Yang R, Muir K, Lophatananon A, Stewart-Brown S, Siriwanarangsan P, Hartman M, Miao H, Chia KS, Chan CW, Fasching PA, Hein A, Beckmann MW, Haeberle L, Brenner H, Dieffenbach AK, Arndt V, Stegmaier C, Ashworth A, Orr N, Schoemaker MJ, Swerdlow AJ, Brinton L, Garcia-Closas M, Zheng W, Halverson SL, Shrubsole M, Long J, Goldberg MS, Labrèche F, Dumont M, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Grip M, Brauch H, Hamann U, Brüning T; GENICA Network, Radice P, Peterlongo P, Manoukian S, Bernard L, Bogdanova NV, Dörk T, Mannermaa A, Kataja V, Kosma VM, Hartikainen JM, Devilee P, Tollenaar RA, Seynaeve C, Van Asperen CJ, Jakubowska A, Lubinski J, Jaworska K, Huzarski T, Sangrajrang S, Gaborieau V, Brennan P, McKay J, Slager S, Toland AE, Ambrosone CB, Yannoukakos D, Kabisch M, Torres D, Neuhausen SL, Anton-Culver H, Luccarini C, Baynes C, Ahmed S, Healey CS, Tessier DC, Vincent D, Bacot F, Pita G, Alonso MR, Álvarez N, Herrero D, Simard J, Pharoah PP, Kraft P, Dunning AM, Chenevix-Trench G, Hall P, Easton DF. Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. Nat Genet 2015;47:373-80.
- Micheli A, Muti P, Secreto G, Krogh V, Meneghini E, Venturelli E, Sieri S, Pala V, Berrino F. Endogenous sex hormones and subsequent breast cancer in premenopausal women. Int J Cancer 2004;112:312-8.
- Michels KB, Terry KL, Willett WC. Longitudinal study on the role of body size in premenopausal breast cancer. Arch Intern Med 2006;166:2395.

- Michels KB, Xue F, Colditz GA, Willett WC. Induced and spontaneous abortion and incidence of breast cancer among young women: a prospective cohort study. Arch Intern Med 2007; 167:814.
- Miki Y, Swensen J, Shattuck-Eidens D, *et al*. . A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266:66-71.
- Miller CP. SERMs: evolutionary chemistry, revolutionary biology. Curr Pharm Des 2002;8:2089-111.
- Miller, Kristi A; Yoshikawa Daniel M; McConnell Ian R; Clark Robin; Schild David; Albala Joanna S. RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51. J Biol Chem 2002;277:8406–11.
- Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, Smith LV, Labbok MH, Geradts J, Bensen JT, Jackson S, Nyante S, Livasy C, Carey L, Earp HS, Perou CM. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008;109:123-39.
- Milne RL, Goode EL, García-Closas M, Couch FJ, Severi G, Hein R, Fredericksen Z, Malats N, Zamora MP, Arias Pérez JI, Benítez J, Dörk T, Schürmann P, Karstens JH, Hillemanns P, Cox A, Brock IW, Elliot G, Cross SS, Seal S, Turnbull C, Renwick A, Rahman N, Shen CY, Yu JC, Huang CS, Hou MF, Nordestgaard BG, Bojesen SE, Lanng C, Grenaker Alnæs G, Kristensen V, Børrensen-Dale AL, Hopper JL, Dite GS, Apicella C, Southey MC, Lambrechts D, Yesilyurt BT, Floris G, Leunen K, Sangrajrang S, Gaborieau V, Brennan P, McKay J, Chang-Claude J, Wang-Gohrke S, Radice P, Peterlongo P, Manoukian S, Barile M, Giles GG, Baglietto L, John EM, Miron A, Chanock SJ, Lissowska J, Sherman ME, Figueroa JD, Bogdanova NV, Antonenkova NN, Zalutsky IV, Rogov YI, Fasching PA, Bayer CM, Ekici AB, Beckmann MW, Brenner H, Müller H, Arndt V, Stegmaier C, Andrulis IL, Knight JA, Glendon G, Mulligan AM, Mannermaa A, Kataja V, Kosma VM, Hartikainen JM, Meindl A, Heil J, Bartram CR, Schmutzler RK, Thomas GD, Hoover RN, Fletcher O, Gibson LJ, dos Santos Silva I, Peto J, Nickels S, Flesch-Janys D, Anton-Culver H, Ziogas A, Sawyer E, Tomlinson I, Kerin M, Miller N, Schmidt MK, Broeks A, Van 't Veer LJ, Tollenaar RA, Pharoah PD, Dunning AM, Pooley KA, Marme F, Schneeweiss A, Sohn C, Burwinkel B, Jakubowska A, Lubinski J, Jaworska K, Durda K, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Kraft P, Lindstrom S, Chen X, Beesley J, Hamann U, Harth V, Justenhoven C; GENICA Network, Winqvist R, Pylkäs K, Jukkola-Vuorinen A, Grip M, Hooning M, Hollestelle A, Oldenburg RA, Tilanus-Linthorst M, Khusnutdinova E, Bermisheva M, Prokofieva D, Farahtdinova A, Olson JE, Wang X, Humphreys MK, Wang Q, Chenevix-Trench G; kConFab Investigators; AOCS Group, Easton DF. Confirmation of 5p12 as a susceptibility locus for progesterone-receptor-positive, lower grade breast cancer. Cancer Epidemiol Biomarkers Prev 2011;20:2222-31.
- Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. J Natl Cancer Inst 2004;96:1856.
- Monninkhof EM, Velthuis MJ, Peeters PH, *et al*. Effect of exercise on postmenopausal sex hormone levels and role of body fat: a randomized controlled trial. J Clin Oncol 2009; 27:4492.
- Moorman PG, Grubber JM, Millikan RC, Newman B. Association between non-steroidal antiinflammatory drugs (NSAIDs) and invasive breast cancer and carcinoma in situ of the breast. Cancer Causes Control 2003;14:915-22.

- Moorman PG, Havrilesky LJ, Gierisch JM, *et al*. Oral contraceptives and risk of ovarian cancer and breast cancer among high-risk women: a systematic review and meta-analysis. J Clin Oncol 2013;31:4188.
- Moraitis D, Du B, De Lorenzo MS, Boyle JO, Weksler BB, Cohen EG, Carew JF, Altorki NK, Kopelovich L, Subbara- maiah K, Dannenberg AJ. Levels of cyclooxygenase-2 are in- creased in the oral mucosa of smokers: evidence for the role of epidermal growth factor receptor and its ligands. Cancer Res 2005;65:664-70.
- Morimoto LM, White E, Chen Z, *et al*. . Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control 2002;13:741.
- Moysich KB, Mettlin C, Piver MS, Natarajan N, Menezes RJ, Swede H. Regular use of analgesic drugs and ovarian cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:903–10.
- Muller-Decker K, Berger I, Ackermann K, Ehemann V, Zoubova S, Aulmann S, Pyerin W, Furstenberger G. Cystic duct dilatations and proliferative epithelial lesions in mouse mammary glands upon keratin 5 promoter-driven overexpression of cyclooxygenase-2. Am J Pathol 2005;166:575–84.
- Mumm JS, Kopan R. Notch signaling: from the outside in. Dev Biol 2000;228:151-65.
- Muscat JE, Stellman SD, Wynder EL. Nonsteroidal antiinflammatory drugs and colorectal cancer. Cancer 1994;74:1847–54.
- Myung SJ, Rerko RM, Yan M, Platzer P, Guda K, Dotson A, Lawrence E, Dannenberg AJ, Lovgren AK, Luo G, *et al*. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proc Natl Acad Sci USA 2006;103:12098–102.
- Naderi A, Teschendorff AE, Barbosa-Morais NL, Pinder SE, Green AR, Powe DG, Robertson JF, Aparicio S, Ellis IO, Brenton JD, Caldas C. A gene-expression signature to predict survival in breast cancer across independent data sets. Oncogene 2007;26:1507-16.
- Nakatsugi S, Ohta T, Kawamori T, Mutoh M, Tanigawa T, Watanabe K, Sugie S, Sugimura T, Wakabayashi K. Chemoprevention by nimesulide, a selective cyclooxygenase-2 inhibitor, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-inducedmammary gland carcinogenesis in rats. Jpn J Cancer Res 2000;91:886–92.
- Nakopoulou L, Mylona E, Papadaki I, Kapranou A, Giannopoulou I, Markaki S, Keramopoulos A. Overexpression of cyclooxygenase-2 is associated with a favorable prognostic phenotype in breast carcinoma. Pathobiology 2005;72:241-9.
- Nam S, Chang HR, Jung HR, Gim Y, Kim NY, Grailhe R, Seo HR, Park HS, Balch C, Lee J, Park I, Jung SY, Jeong KC, Powis G, Liang H, Lee ES, Ro J, Kim YH. A pathway-based approach for identifying biomarkers of tumor progression to trastuzumab-resistant breast cancer. Cancer Lett 2015;356:880-90.
- Namiranian N, Moradi-Lakeh M, Razavi-Ratki SK, Doayie M, Nojomi M. Risk factors of breast cancer in the eastern mediterranean region: a systematic review and meta-analysis. Asian Pac J Cancer Prev 2014;159535-41.
- Nartey T, Moran H, Marin T, Arcaro KF, Anderton DL, Etkind P, Holland JF, Melana SM, Pogo BG. Human Mammary Tumor Virus (HMTV) sequences in human milk. Infect Agent Cancer. 2014;9:20.

- Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties, and functions. Physiol Rev 1999;79:1193–1226.
- Neugut AI, Rosenberg DJ, Ahsan H, Jacobson JS, Wahid N, Hagan M, Rahman MI, Khan ZR, Chen L, Pablos-Mendez A, Shea S. Association between coronary heart disease and cancers of the breast, prostate, and colon. Cancer Epidemiol Biomarkers Prev 1998;7:869-73.
- Nickell WB, Skelton J. Breast fat and fallacies: more than 100 years of anatomical fantasy. J Hum Lact 2005;21:126-30.
- NIH Halts Use of COX-2 Inhibitor in Large Cancer Prevention Trial 2004.
- Nupponen NN, Kakkola L, Koivisto P, Visakorpi T. Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998;153:141-8.
- Nyante SJ, Gammon MD, Kaufman JS, Bensen JT, Lin DY, Barnholtz-Sloan JS, Hu Y, He Q, Luo J, Millikan RC. Genetic variation in estrogen and progesterone pathway genes and breast cancer risk: an exploration of tumor subtype-specific effects. Cancer Causes Control 2015;26:121-31.
- Olsson HL, Ingvar C, Bladström A. Hormone replacement therapy containing progestins and given continuously increases breast carcinoma risk in Sweden. Cancer 2003;97:1387.
- Oral-contraceptive use and the risk of breast cancer. The Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development. N Engl J Med 1986;315:405.
- Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2) Cell 1996;87:803–9.
- Ostroumova E, Preston DL, Ron E, *et al*. . Breast cancer incidence following low-dose rate environmental exposure: Techa River Cohort, 1956-2004. Br J Cancer 2008;99:1940.
- Ou SM, Chen YT, Chao PW, Lee YJ, Liu CJ, Yeh CM, Chen TJ, Chen TW, Yang WC, Li SY. Nonsteroidal antiinflammatory drug use is associated with cancer risk reduction in chronic dialysis patients. Kidney Int 2013;84:198-205.
- Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. BMJ 1989;299:1247-50.
- Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gas- trointestinal hypertrophy. Nat Med 2002;8:289-93.
- Palmer JR, Boggs DA, Wise LA, Ambrosone CB, Adams-Campbell LL, Rosenberg L. Parity and lactation in relation to estrogen receptor negative breast cancer in African American women. Cancer Epidemiol Biomarkers Prev 2011;20:1883-91.
- Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohsnitter W, *et al*. Prenatal diethylstilbestrol exposure and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2006;15:1509-14.
- Paoletti X, Clavel-Chapelon F. Induced and spontaneous abortion and breast cancer risk: results from the E3N cohort study. Int J Cancer 2003;106:270.

- Park KJ, Krishnan V, O'Malley BW, *et al*. Formation of an IKKalpha-dependent transcription complex is required for estrogen receptor-mediated gene activation. Mol Cell 2005;18:71–82.
- Park WC and Jordan VC Selective estrogen receptor modulators (SERMs) and their roles in breast cancer prevention Trends Mol Med 2002;8:82-88
- Parrett M, Harris R, Joarder F, Ross M, Clausen K, Robertson F. Cyclooxygenase-2 gene expression in human breast cancer. Int J Oncol 1997;10:503-7.
- Partridge RK, Hähnel R. Prolactin receptors in human breast carcinoma. Cancer 1979;43:643-6
- Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M, Maloche C. Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. JCEM 1996;81:1460-4.
- Pastor-Barriuso R, Ascunce N, Ederra M, Erdozáin N, Murillo A, Alés-Martínez JE, Pollán M. Recalibration of the Gail model for predicting invasive breast cancer risk in Spanish women: a population-based cohort study. Breast Cancer Res Treat 2013;138:249-5
- Patrono C, Coller B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsh J, Roth G. Platelet-active drugs - The relationships among dose, effectiveness, and side effects. Chest 2001;119:39S–63S.
- Peleg II, Maibach HT, Brown SH, Wilcox CM. Aspirin and nonsteroidal antiinflammatory drug use and the risk of subsequent colorectal cancer. Arch Intern Med 1994;154:394–9.
- Perillo B, Di Santi A, Cernera G, *et al*. Phosphorylation of H3 serine 10 by IKKα governs cyclical production of ROS in estrogen-induced transcription and ensures DNA wholeness. Cell Death Differ 2014;21:1503.
- Perillo B, Ombra MN, Bertoni A, *et al*. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. Science 2008;319:202–6.
- Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
- Perrone G, Santini D, Vincenzi B, Zagami M, La Cesa A, Bianchi A, Altomare V, Primavera A, Battista C, Vetrani A, Tonini G, Rabitti C. COX-2 expression in DCIS: correlation with VEGF, HER-2/neu, prognostic molecular markers and clinicopathological features. Histopathology 2005;46:561-8.
- Peshkin BN, Isaacs C. Characteristics of hereditary breast and ovarian cancer syndrome. En: UpToDate, Basow, DS (Ed), UpToDate, Waltham, MA, 2012.
- Petersen C, Petersen S, Milas L, Lang FF, Tofilon PJ. En- hancement of intrinsic tumor cell radiosensitivity induced by a selective cyclooxygenase-2 inhibitor. Clin Cancer Res 2000;6: 2513-20.
- Peto R, Gray R, Collins R, Wheatley K, Hennekens C, Jamrozik K, Warlow C, Hafner B, Thompson E, Norton S. Randomised trial of prophylactic daily aspirin in British male doctors. BMJ 1988;296:313–16.
- Philip M, Rowley DA, Schreiber H. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol 2004;14:433-39.

- Phipps AI, Chlebowski RT, Prentice R, McTiernan A, Wactawski-Wende J, Kuller LH, Adams-Campbell LL, Lane D, Stefanick ML, Vitolins M, Kabat GC, Rohan TE, Li CI. Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. J Natl Cancer Inst 2011;103:470-7.
- Phoenix KN, Vumbaca F, Fox MM, Evans R, Claffey KP. Dietary energy availability affects primary and metastatic breast cancer and metformin efficacy. Breast Cancer Res Treat 2010;123:333–44.
- Plastaras JP, Guengerich FP, Nebert DW, Marnett LJ. Xeno- biotic-metabolizing cytochromes P450 convert prostaglandin endoperoxide to hydroxyheptadecatrienoic acid and the mu- tagen, malondialdehyde. J Biol Chem 2000;275:11784-90.
- Pockaj BA, Basu GD, Pathangey LB, Gray RJ, Hernandez JL, Gendler SJ, Mukherjee P. Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and prostaglandin E2 secretion in patients with breast cancer. Ann Surg Oncol 2004;11:328-39.
- Polakis P. The oncogenic activation of beta-catenin. Curr Opin Genet Dev 1999;9:15-21.
- Pollak M. IGF-I physiology and breast cancer. Recent Results Cancer Res 1998;152:63-70.
- Pollak MN, Chapman JW, Pritchard KI, Krook JE, Dhaliwal HS, Vandenberg TA, *et al*. Tamoxifen versus tamoxifen plus octreotideLARas adjuvant therapy for early-stage breast cancer in postmenopausal women: update of NCIC CTG MA14 trial. J Clin Oncol 2010;28:542.
- Pollak MN. Endocrine effects of IGF-I on normal and transformed breast epithelial cells: potential relevance to strategies for breast cancer treatment and prevention. Breast Cancer Res Treat 1998;47:209-17.
- Pollán M, Ascunce N, Ederra M, et al. Mammographic density and risk of breast cancer according to tumor characteristics and mode of detection: a Spanish population-based case-control study. Breast Cancer Research 2013;15:R9.
- Prat, A., Parker, J., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J., He, X., Perou, C. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12:R68
- Prentice RL, Chlebowski RT, Stefanick ML, *et al*. Conjugated equine estrogens and breast cancer risk in the Women's Health Initiative clinical trial and observational study. Am J Epidemiol 2008;167:1407.
- Prentice RL, Chlebowski RT, Stefanick ML, *et al*. Estrogen plus progestin therapy and breast cancer in recently postmenopausal women. Am J Epidemiol 2008;167:1207.
- Prentice RL. Postmenopausal hormone therapy and the risks of coronary heart disease, breast cancer, and stroke. Semin Reprod Med 2014;32:419-25.
- Prosperi JR, Robertson FM. Cyclooxygenase-2 directly regulates gene expression of P450 Cyp19 aromatase promoter regions pII, pI.3 and pI.7 and estradiol production in human breast tumor cells. Prostaglandins Other Lipid Mediat 2006;81:55–70.
- Pukkala E, Kesminiene A, Poliakov S, *et al*. Breast cancer in Belarus and Ukraine after the Chernobyl accident. Int J Cancer 2006;119:651.

- Quigley DA, Fiorito E, Nord S, Van Loo P, Alnæs GG, Fleischer T, Tost J, Moen Vollan HK, Tramm T, Overgaard J, Bukholm IR, Hurtado A, Balmain A, Børresen-Dale AL, Kristensen V. The 5p12 breast cancer susceptibility locus affects MRPS30 expression in estrogen-receptor positive tumors. Mol Oncol 2014;8:273-84.
- Rahman N, Seal S, Thompson D, *et al*. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 2007;39:165-7.
- Rahme E, Ghosn J, Dasgupta K, Rajan R, Hudson M. Association between frequent use of nonsteroidal antiinflammatory drugs and breast cancer. BMC Cancer 2005;5:159.
- Rahme E, Joseph L, Kong SX, Watson DJ, LeLorier J. Gastrointestinal health care resource use and costs associated with nonsteroidal antiinflammatory drugs versus acetaminophen: retrospective cohort study of an elderly population. Arthritis Rheum 2000;43:917–24.
- Rapp UR, Marshall TH. Cell surface receptors for endogenous mouse type C viral glycoproteins and epidermal growth factor: tissue distribution in vivo and possible participation in specific cell-cell interaction. J Supramol Struct 1981;14:343–52.
- Ratnasinghe LD, Graubard BI, Kahle L, Tangrea JA, Taylor PR, Hawk E. Aspirin use and mortality from cancer in a prospective cohort study. Anticancer Res 2004;24:3177-84.
- Rea S, Eisenhaber F, O'Carroll D, *et al*. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 2000;406:593–9.
- Ready A, Velicer CM, McTiernan A, White E. NSAID use and breast cancer risk in the VITAL cohort. Breast Cancer Res Treat 2008;109:533-43.
- Rebbeck TR, Friebel T, Wagner T, Lynch HT, Garber JE, Mary B. Daly, Isaacs C, Olopade OI, Neuhausen SL, Van't Veer L, Eeles R, Evans DG, Tomlinson G, Matloff E, Narod SA, Eisen A, Domchek S, Armstrong K and Weber BL. Effect of Short-Term Hormone Replacement Therapy on Breast Cancer Risk Reduction After Bilateral Prophylactic Oophorectomy in BRCA1and BRCA2 Mutation Carriers: The PROSE Study Group. Journal of Clinical Oncology 2005;23:7804-10.
- Reddy BS. Studies with the azoxymethanerat preclinical model for assessing colon tumor development and chemoprevention. Environ Mol Mutagen 2004;44:26–35.
- Reeves GK, Kan SW, Key T, *et al*. Breast cancer risk in relation to abortion: Results from the EPIC study. Int J Cancer 2006;119:1741.
- Reiss M, Barcellos-Hoff MH. Transforming growth factor-beta in breast cancer: a working hypothesis. Breast Cancer Res Treat 1997;45:81-95.
- Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346-53.
- Rice MC, Smith ST, Bullrich F, Havre P, Kmiec EB. Isolation of human and mouse genes based on homology to REC2, a recombinational repair gene from the fungus Ustilago maydis. Proc Natl Acad Sci USA 1997;94:7417–22.
- Richards JA, Brueggemeier RW. Interactions of cyclooxy- genase and aromatase pathways in normal and malignant breast cells. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002:205-24 .

- Ripperger T, Gadzicki D, Meindl A, Schlegelberger B. Breast cancer susceptibility: current knowledge and implications for genetic counselling. Eur J Hum Genet 2009;17:722-31.
- Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 2002;62:632-35.
- Robertson FM, Parrett ML, Joarder FS, Ross M, Abou-Issa HM, Alshafie G, Harris RE. Ibuprofen-induced inhibition of cyclooxygenase isoform gene expression and regression of rat mammary carcinomas. Cancer Lett 1998;122:165-75.
- Rodrigues LAG, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology 2001;12:88–93.
- Rogan EG, Badawi AF, Devanesan PD, Meza JL, Edney JA, West WW, Higginbotham SM, Cavalieri EL. Relative imbal- ances in estrogen metabolism and conjugation in breast tis- sue of women with carcinoma: potential biomarkers of sus- ceptibility to cancer. Carcinogenesis 2003;24:697-702.
- Romieu I, Berlin JA, Colditz G. Oral contraceptives and breast cancer Review and meta-analysis. Cancer 1990;66:2253-63.
- Rose DP, Connolly JM. Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 1999;83:217-44.
- Rose DP, Vona-Davis L. Biochemical and molecular mecha- nisms for the association between obesity, chronic inflam- mation, and breast cancer. Biofactors 2014;40:1-12.
- Rose-Hellekant TA, Arendt LM, Schroeder MD, Gilchrist K, Sandgren EP, Schuler LA. Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. Oncogene 2003;22:4664-74.
- Rosenberg L, Louik C, Shapiro S. Nonsteroidal antiinflammatory drug use and reduced risk of large bowel carcinoma. Cancer 1998;82:2326–33.
- Rosenberg L, Palmer JR, Zauber AG, Warshauer ME, Stolley PD, Shapiro S. A hypothesis: nonsteroidal antiinflammatory drugs reduce the incidence of large-bowel cancer. J Natl Cancer Inst 1991;83:355–8.
- Rosenberg L. Nonsteroidal antiinflammatory drugs and cancer. Prev Med. 1995;24(2):107-9.
- Rosner B, Colditz GA, Willett WC. Reproductive risk factors in a prospective study of breast cancer: the Nurses' Health Study. Am J Epidemiol 1994;139:819-35.
- Ross RK, Paganini-Hill A, Wan PC, Pike MC. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. J Natl Cancer Inst 2000;92:328.
- Rossouw JE, Anderson GL, Prentice RL, *et al*. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321.
- Rostom A, Dubé C, Lewin G, Tsertsvadze A, Barrowman N, Code C, Sampson M, Moher D; U.S. Preventive Services Task Force. Nonsteroidal antiinflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med 2007;146:376-89.

- Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ. Comparative study of human and rat mammary tumorigenesis. Lab Invest 1990;62:244-78.
- Salaspuro M. Acetaldehyde as a common denominator and cumulative carcinogen in digestive tract cancers. Scand J Gastroenterol 2009;44:912-25.
- Santen RJ, Mansel R. Benign breast disorders. N Engl J Med 2005;353:275-85.
- Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L, Hoover R. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. JAMA 2000;283:485-91.
- Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994;5:138-46.
- Schrieber H, Rowley DA. Inflammation and cancer. In: JI Gallin, R Snyderman, eds. Inflammation: Basic Principles and Clinical Correlates, third ed. Philadelphia: Lippincott Williams & Wilkins, 1999: 1117-29.
- Sears RC. The life cycle of C-myc: from synthesis to degradation. Cell Cycle 2004;3:1133-7.
- Seksenyan A, Kadavallore A, Walts AE, de la Torre B, Berel D, Strom SP, Aliahmad P, Funari VA, Kaye J. TOX3 is expressed in mammary ER + epithelial cells and regulates ER target genes in luminal breast cancer. BMC Cancer 2015;15:22.
- Seno H, Oshima M, Ishikawa TO, Oshima H, Takaku K, Chiba T, Narumiya S, Taketo MM. Cyclooxygenase 2- and prostaglandin E_2 receptor EP_2-dependent angiogenesis in $Apc^{\Delta 716}$ mouse intestinal polyps. Cancer Res 2002;62:506–11.
- Shamoo Y. Structural insights into BRCA2 function. Curr Opin Struc Biol 2003; 13 (2): 206-11.
- Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. Science. 2002;295:2465-8.
- Sharma RA, Gescher A, Plastaras JP, Leuratti C, Singh R, Gallacher-Horley B, Offord E, Marnett LJ, Steward WP, Plummer SM. Cyclooxygenase-2, malondialdehyde and py- rimidopurinone adducts of deoxyguanosine in human colon cells. Carcinogenesis 2001;22: 1557-60.
- Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA. Nested case-control study of the effects of non-steroidal antiinflammatory drugs on breast cancer risk and stage. Br J Cancer 2000;83:112-20.
- Shen J, Gammon MD, Terry MB, Teitelbaum SL, Neugut AI, Santella RM. Genetic polymorphisms in the cyclooxygenase-2 gene, use of nonsteroidal antiinflammatory drugs, and breast cancer risk. Breast Cancer Res 2006;8:R71.
- Shiff SJ, Rigas B. The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal antiinflamma- tory drugs (NSAIDs). J Exp Med 1999;190:445-50.
- Shim JY, An HJ, Lee YH, Kim SK, Lee KP, Lee KS. Overexpression of cyclooxygenase-2 is associated with breast carcinoma and its poor prognostic factors. Mod Pathol 2003;16:1199-204.
- Shim V, Gauthier ML, Sudilovsky D, Mantei K, Chew KL, Moore DH, Cha I, Tlsty TD, Esserman LJ. Cyclooxygenase-2 expression is related to nuclear grade in ductal carcinoma in situ and is increased in its normal adjacent epithelium. Cancer Res 2003;63:2347-50.

- Shimizu K, Chiba S, Hosoya N, Kumano K, Saito T, Kurokawa M, Kanda Y, Hamada Y, Hirai H. Binding of Delta1, Jagged1, and Jagged2 to Notch2 rapidly induces cleavage, nuclear translocation, and hyperphosphorylation of Notch2. Mol Cell Biol 2000;20:6913-22.
- Shiozaki EN, Gu L, Yan N, Shi Y. Structure of the BRCT repeats of BRCA1 bound to a BACH1 phosphopeptide: implications for signaling. Mol Cell 2004;14:405-12.
- Shugart YY, Cour C, Renard H, *et al*. . Linkage analysis of 56 multiplex families excludes the Cowden disease gene PTEN as a major contributor to familial breast cancer. J Med Genet 1999;36:720-1.
- Siegel PM, Ryan ED, Cardiff RD, Muller WJ. Elevated expression of activated forms of Neu/ErbB-2 and ErbB-3 are involved in the induction of mammary tumors in transgenic mice: implications for human breast cancer. Embo J 1999;18:2149–64.
- Siemes C, Visser LE, Coebergh JW, Hofman A, Uitterlinden AG, Stricker BH. Protective effect of NSAIDs on cancer and influence of COX-2 C(-765G) genotype. Curr Cancer Drug Targets 2008;8:753-64.
- Sieri S, Krogh V, Bolelli G, *et al*. . Sex hormone levels, breast cancer risk, and cancer receptor status in postmenopausal women: the ORDET cohort. Cancer Epidemiol Biomarkers Prev 2009;18:169.
- Sigurdsson, S; Van Komen S; Bussen W; Schild D; Albala J S; Sung P. Mediator function of the human Rad51B-Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. Genes Dev 2001;15:3308–18.
- Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal antiinflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA 2000;284:1247–55.
- Simpson ER, Brown KA. Minireview: Obesity and breast cancer: a tale of inflammation and dysregulated metabolism. Mol Endocrinol 2013;27:715-5.
- Singh B, Berry JA, Shoher A, Ramakrishnan V, Lucci A. COX-2 overexpression increases motility and invasion of breast cancer cells. Int J Oncol 2005;26:1393-9.
- Singh-Ranger G, Kirkpatrick KL, Clark GM, Mokbel K. Cyclo-oxygenase-2 (COX-2) mRNA expression correlates with progesterone receptor positivity in human breast cancer. Curr Med Res Opin 2003;19:131-4.
- Singh-Ranger G, Salhab M, Mokbel K. The role of cyclooxygenase-2 in breast cancer: review. Breast Cancer Res Treat 2008;109:189-98.
- Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. JAMA 2001;286:2143.
- Sivula A, Talvensaari-Mattila A, Lundin J, Joensuu H, Ha- glund C, Ristimäki A, Turpeenniemi-Hujanen T. Association of cyclooxygenase-2 and matrix metalloproteinase-2 expres- sion in human breast cancer. Breast Cancer Res Treat 2005;89:215-20.

- Slattery ML, Curtin K, Baumgartner R, Sweeney C, Byers T, Giuliano AR, Baumgartner KB, Wolff RR. IL6, aspirin, nonsteroidal antiinflammatory drugs, and breast cancer risk in women living in the southwestern United States. Cancer Epidemiol Biomarkers Prev 2007;16:747-55.
- Smalley W, Ray WA, Daugherty J, Griffin MR. Use of nonsteroidal antiinflammatory drugs and incidence of colorectal cancer: a population-based study. Arch Intern Med 1999;159:161–6.
- Smith CC, Taylor HS. Xenoestrogen exposure imprints expression of genes (Hoxa10) required for normal uterine development. FASEB J. 2007;21:239-46.
- Söderlund L K, Asklid A, Fornander T, Stenmark Askmalm M. The RAD51 135G>C polymorphism is related to the effect of adjuvant therapy in early breast cancer. J Cancer Res Clin Oncol 2015;141:797-804.
- Soh JW, Weinstein IB. Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. Prog Exp Tumor Res 2003;37:261–285.
- Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 2005;352:1071–1080.
- Somyajit K, Subramanya S, Nagaraju G. RAD51C: a novel cancer susceptibility gene is linked to Fanconi anemia and breast cancer. Carcinogenesis 2010;31:2031–8.
- Son BH, Kim MK, Yun YM, Kim HJ, Yu JH, Ko BS, Kim H, Ahn SH. Genetic polymorphism of ESR1 rs2881766 increases breast cancer risk in Korean women. J Cancer Res Clin Oncol 2015;141:633-45.
- Sørensen HT, Friis S, Nørgård B, Mellemkjaer L, Blot WJ, McLaughlin JK, Ekbom A, Baron JA. Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study. Br J Cancer 2003;88:1687-92.
- Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, Van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98:10869–74.
- Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2000;89:2637-45.
- Spizzo G, Gastl G, Wolf D, Gunsilius E, Steurer M, Fong D, Amberger A, Margreiter R, Obrist P. Correlation of COX-2 and Ep-CAM overexpression in human invasive breast cancer and its impact on survival. Br J Cancer 2003;88:574–8.
- Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA, Masson G, Jakobsdottir M, Thorlacius S, Helgason A, Aben KK, Strobbe LJ, Albers-Akkers MT, Swinkels DW, Henderson BE, Kolonel LN, Le Marchand L, Millastre E, Andres R, Godino J, Garcia-Prats MD, Polo E, Tres A, Mouy M, Saemundsdottir J, Backman VM, Gudmundsson L, Kristjansson K, Bergthorsson JT, Kostic J, Frigge ML, Geller F, Gudbjartsson D, Sigurdsson H, Jonsdottir T, Hrafnkelsson J, Johannsson J, Sveinsson T, Myrdal G, Grimsson HN, Jonsson T, von Holst S, Werelius B, Margolin S, Lindblom A, Mayordomo JI, Haiman CA, Kiemeney LA, Johannsson OT, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet 2007;39:865-9.

- Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, Jonsson GF, Jakobsdottir M, Bergthorsson JT, Gudmundsson J, Aben KK, Strobbe LJ, Swinkels DW, van Engelenburg KC, Henderson BE, Kolonel LN, Le Marchand L, Millastre E, Andres R, Saez B, Lambea J, Godino J, Polo E, Tres A, Picelli S, Rantala J, Margolin S, Jonsson T, Sigurdsson H, Jonsdottir T, Hrafnkelsson J, Johannsson J, Sveinsson T, Myrdal G, Grimsson HN, Sveinsdottir SG, Alexiusdottir K, Saemundsdottir J, Sigurdsson A, Kostic J, Gudmundsson L, Kristjansson K, Masson G, Fackenthal JD, Adebamowo C, Ogundiran T, Olopade OI, Haiman CA, Lindblom A, Mayordomo JI, Kiemeney LA, Gulcher JR, Rafnar T, Thorsteinsdottir U, Johannsson OT, Kong A, Stefansson K. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet. 2008;40:703-6.
- Stahlberg C, Pedersen AT, Lynge E, *et al*. Increased risk of breast cancer following different regimens of hormone replacement therapy frequently used in Europe. Int J Cancer 2004; 109:721.
- Stanford JL, Thomas DB. Exogenous progestins and breast cancer. Epidemiol Rev 1993; 15:98-107.
- Staveley-O'Carroll K, Sotomayor E, Montgomery J, Borrello I, Hwang L, Fein S, Pardoll D, Levitsky H. Induction of anti- gen-specific T cell anergy: An early event in the course of tu- mor progression. Proc Natl Acad Sci USA 1998;95:1178-83.
- Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, *et al*. . The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–52.
- Stevenson DD. Aspirin and NSAID sensitivity. Immunol Allergy Clin North Am. 2004 Aug;24(3):491-505, vii
- Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Benbrahim-Tallaa L, Cogliano V. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol 2007;8:1065-6.
- Stuebe AM, Willett WC, Xue F, Michels KB. Lactation and incidence of premenopausal breast cancer: a longitudinal study. Arch Intern Med 2009;169:1364.
- Stürmer T, Glynn RJ, Lee IM, Manson JE, Buring JE, Hennekens CH. Aspirin use and colorectal cancer: post-trial follow-up data from the Physicians' Health Study. Ann Intern Med 1998;128:713–20.
- Stygar D, Muravitskaya N, Eriksson B, Eriksson H, Sahlin L. Effects of SERM (selective estrogen receptor modulator) treatment on growth and proliferation in the rat uterus. Reprod Biol Endocrinol 2003;1:40.
- Suarez F, Mahlaoui N, Canioni D, Andriamanga C, Dubois d'Enghien C, Brousse N, Jais JP, Fischer A, Hermine O, Stoppa-Lyonnet D. Incidence, presentation, and prognosis of malignancies in ataxia-telangiectasia: a report from the French national registry of primary immune deficiencies. J Clin Oncol 2015;33:202-8.
- Subbaramaiah K, Chung WJ, Dannenberg AJ. Ceramide regulates the transcription of cyclooxygenase-2. Evidence for involvement of extracellular signal-regulated kinase/c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways. J Biol Chem 1998;273: 32943-9 .

- Subbaramaiah K, Dannenberg AJ. Cyclooxygenase 2: a mo- lecular target for cancer prevention and treatment. Trends Pharmacol Sci 2003;24:96-102.
- Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, Zhou XK, Blaho VA, Hla T, Yang P, Kopelovich L, Hudis CA, Dannenberg AJ. Obesity is associated with in- flammation and elevated aromatase expression in the mouse mammary gland. Cancer Prev Res 2011;4: 329-46.
- Subbaramaiah K, Howe LR, Port ER, Brogi E, Fishman J, Liu CH, Hla T, Hudis C, Dannenberg AJ. HER-2/neu status is a determinant of mammary aromatase activity in vivo: evidence for a cyclooxygenase-2-dependent mechanism. Cancer Res 2006;66:5504–11.
- Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, Kopelovich L, Hudis CA, Dannenberg AJ. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. Cancer Discov 2012;2:356-365.
- Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. J Biol Chem 2002;277:18649-57.
- Sugimoto T, Bartholomeusz C, Tari AM, Ueno NT. Adeno- virus type 5 E1A-induced apoptosis in COX-2-overexpress- ing breast cancer cells. Breast Cancer Res 2007;9:R41.
- Suwaki N, Klare K, Tarsounas M. RAD51 paralogs: roles in DNA damage signal-ling, recombinational repair and tumorigenesis. Semin Cell Dev Biol 2011;22:898–905.
- Swede H, Mirand AL, Menezes RJ, Moysich KB. Association of regular aspirin use and breast cancer risk. Oncology 2005;68:40-7.
- Szabo CI, King MC. Population Genetics of BRCA1 and BRCA2. Am J Hum Genet 1997;60:1013-20.
- Takeda H, Sonoshita M, Oshima H, Sugihara K, Chulada PC, Langenbach R, Oshima M, Taketo MM. Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis. Cancer Res 2003;63:4872–7.
- Takeshita E, Osanai T, Higuchi T, Soumaoro LT, Sugihara K. Elevated cyclooxygenase-2 expression is associated with histological grade in invasive ductal breast carcinoma. J Med Dent Sci 2005;52:189-93.
- Takkouche B, Regueira-Méndez C, Etminan M. Breast cancer and use of nonsteroidal antiinflammatory drugs: a meta-analysis. J Natl Cancer Inst 2008;100:1439-47.
- Tamarkin L, Cohen M, Roselle D, Reichert C, Lippman M, Chabner B. Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracen-induced mammary tumors in the rat. Cancer Res 1981;41:4432-36.
- Tamimi RM, Byrne C, Colditz GA, Hankinson SE. Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 2007; 99:1178.Barlow WE, White E, Ballard-Barbash R, Vacek PM, Titus-Ernstoff L, Carney PA, Tice JA, Buist DS, Geller BM, Rosenberg R, Yankaskas BC, Kerlikowske K. Prospective breast cancer risk prediction model for women undergoing screening mammography. J Natl Cancer Inst 2006 Sep 6; 98:1204-14.

- Tamimi RM, Hankinson SE, Chen WY, Rosner B, Colditz GA. Combined estrogen and testosterone use and risk of breast cancer in postmenopausal women. Arch Intern Med 2006;166:1483-89.
- Tan KB, Yong WP, Putti TC. Cyclooxygenase-2 expression: a potential prognostic and predictive marker for high-grade ductal carcinoma in situ of the breast. Histopathology 2004; 44:24-28.
- Tang L, Xu J, Wei F, Wang L, Nie WW, Chen LB, Guan XX. Association of STXBP4/COX11 rs6504950 (G>A) polymorphism with breast cancer risk: evidence from 17,960 cases and 22,713 controls. Arch Med Res 2012;43:383-8.
- Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, Subbaramaiah K, Dannenberg AJ, Neugut AI. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. JAMA 2004;291:2433-40.
- Thomas DB. Oral contraceptives and breast cancer: review of the epidemiologic literature. Contraception.1991;43:597-642.
- Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, Cox DG, Hankinson SE, Hutchinson A, Wang Z, Yu K, Chatterjee N, Garcia-Closas M, Gonzalez-Bosquet J, Prokunina-Olsson L, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Diver R, Prentice R, Jackson R, Kooperberg C, Chlebowski R, Lissowska J, Peplonska B, Brinton LA, Sigurdson A, Doody M, Bhatti P, Alexander BH, Buring J, Lee IM, Vatten LJ, Hveem K, Kumle M, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover RN, Chanock SJ, Hunter DJ. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet 2009;41:579-84.
- Thomas HV, Key TJ, Allen DS, *et al*. A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. Br J Cancer 1997;75:1075.
- Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control 1997;8:922-8
- Thompson D, Szabo CI, Mangion J, *et al*. Evaluation of linkage of breast cancer to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the Breast Cancer Linkage Consortium. Proc Natl Acad Sci USA 2002;99:827-31.
- Thun MJ, Henley SJ, Patrono C. Nonsteroidal antiinflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94:252-66.
- Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW Jr. Aspirin use and risk of fatal cancer. Cancer Res 1993;53:1322-7.
- Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW Jr. Aspirin use and risk of fatal cancer. Cancer Res 1993;53:1322–7.
- Thun MJ, Namboodiri MM, Heath CW., Jr Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325:1593–6.
- Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, Dunson DB, Rogan EG, Morham SG, Smart RC, *et al*. . Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 2002;62:3395–401.

- Tice JA, O'Meara ES, Weaver DL, Vachon C, Ballard-Barbash R, Kerlikowske K. Benign Breast Disease, Mammographic Breast Density, and the Risk of Breast Cancer. JNCI Journal of the National Cancer Institute 2013;105:1043-49.
- Timoshenko AV, Chakraborty C, Wagner GF, Lala PK. COX-2-mediated stimulation of the lymphangiogenic fac- tor VEGF-C in human breast cancer. Br J Cancer 2006;94:1154-63.
- Tjønneland A, Christensen J, Thomsen BL, *et al*. Hormone replacement therapy in relation to breast carcinoma incidence rate ratios: a prospective Danish cohort study. Cancer 2004;100:2328.
- Tolentino Silva, M, Freire Galvao, T, Ricardo Zimmerman, I, Gomes Pereira, M, Cruz Lopes, L. Non-aspirin Non-steroidal Antiinflammatory Drugs for the Primary Chemoprevention of Non-gastrointestinal Cancer: Summary of Evidence. Curr Pharm Des 2012;18:4047-70.
- Totzke G, Schulze-Osthoff K, Jänicke RU. Cyclooxygenase-2 (COX-2) inhibitors sensitize tumor cells specifically to death receptor-induced apoptosis independently of COX-2 inhibition. Oncogene 2003;22:8021-30.
- Tretli S. Height and weight in relation to breast cancer morbidity and mortality. A prospective study of 570,000 women in Norway. Int J Cancer 1989;44:23-30.
- Trichopoulos D, MacMahon B, Cole P. Menopause and breast cancer risk. J Natl Cancer Inst 1972;48:605-13.
- Tryggvadóttir L, Tulinius H, Eyfjord JE, Sigurvinsson T. Breastfeeding and reduced risk of breast cancer in an Icelandic cohort study. Am J Epidemiol 2001;154:37.
- Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin en- doperoxide synthase 2. Cell 1995;83:493-501.
- Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghoussaini M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W, Eccles D,Evans DG; Breast Cancer Susceptibility Collaboration (UK), Hooning M, Schutte M, van den Ouweland A, Houlston R, Ross G, Langford C, Pharoah PD, Stratton MR, Dunning AM, Rahman N, Easton DF. Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet 2010;42:504-7.
- Tutt A, Ashworth A. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol Med 2002;8:571-6.
- Tworoger SS, Eliassen AH, Sluss P, Hankinson SE. A prospective study of plasma prolactin concentrations and risk of premenopausal and postmenopausal breast cancer. J Clin Oncol 2007;25:1482-8.
- Tworoger SS, Eliassen AH, Zhang X, Qian J, Sluss PM, Rosner BA, Hankinson SE. A 20-year prospective study of plasma prolactin as a risk marker of breast cancer development. Cancer Res 2013;73:4810-9.
- Tworoger SS, Missmer SA, Eliassen AH, *et al*. The association of plasma DHEA and DHEA sulfate with breast cancer risk in predominantly premenopausal women. Cancer Epidemiol Biomarkers Prev 2006;15:967.
- Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 2006;66:2476.

- Ueda T, Yoshida M. HMGB proteins and transcriptional regulation. Biochim Biophys Acta 2010;1799:114-8.
- Vachon CM, Sellers TA, Carlson EE, *et al*. Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. Cancer Res 2007;67:8412.
- Vahteristo P, Yliannala K, Tamminen A, Eerola H, Blomqvist C, Nevanlinna H. BACH1 Ser919Pro variant and breast cancer risk. BMC Cancer 2006;6:19.
- van 't Veer LJ , Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-6.
- van den Brandt PA, Spiegelman D, Yaun SS, *et al*. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152:514.
- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971;231:232-5.
- Vatten LJ, Kvinssland S. Prospective study of height, body mass index and risk of breast cancer. Acta Oncol 1992;31:195-200.
- Verheus M, McKay JD, Kaaks R, Canzian F, Biessy C, Johansson M, Grobbee DE, Peeters PH, van Gils CH. Common genetic variation in the IGF-1 gene, serum IGF-I levels and breast density. Breast Cancer Res Treat 2008;112:109-22.
- Vessey M, Painter R. Oral contraceptive use and cancer. Findings in a large cohort study, 1968-2004. Br J Cancer 2006;95:385.
- Vessey M, Yeates D. Oral contraceptive use and cancer: final report from the Oxford-Family Planning Association contraceptive study. Contraception 2013; 88:678.
- Vinogradova Y, Coupland C, Hippisley-Cox J. Exposure to cyclooxygenase-2 inhibitors and risk of cancer: nested case-control studies. Br J Cancer 2011;105:452-9.
- Virchow R. Aetiologie der neoplastischen Geschwulst/Pathogenie der neoplastischen Geschwulste. In: Die Krankhaften Geschwulste. Berlin: Verlag von August Hirschwald, 1863: 57-101.
- Virchow R. Reizung and Reizbarkeit. Arch Pathol Anat Klin Med 1858; 14: 1-63.
- Visscher DW, Pankratz VS, Santisteban M, Reynolds C, Ristimäki A, Vierkant RA, Lingle WL, Frost MH, Hartmann LC. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. J Natl Cancer Inst 2008;100:421-7.
- Vogel U, Christensen J, Nexø BA, Wallin H, Friis S, Tjønneland A. Peroxisome proliferator-activated [corrected] receptor-gamma2 [corrected] Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes. Carcinogenesis. 2007;28:427-34.
- Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, Bevers TB, Fehrenbacher L, Pajon ER Jr, Wade JL 3rd, Robidoux A, Margolese RG, James J, Lippman SM, Runowicz CD, Ganz PA, Reis SE, McCaskill-Stevens W, Ford LG, Jordan VC, Wolmark N, National Surgical Adjuvant Breast and Bowel Project (NSABP). Effects of tamoxifen vs raloxifene on the

risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA 2006; 295:2727-41.

- Waaler HT, Lund E. Association between body height and death from breast cancer. Br J Cancer 1983;48:149-50.
- Wakefield LM, Roberts AB. TGF-beta signaling: positive and negative effects on tumorigenesis. Curr Opin Genet Dev 2002;12:22-9.
- Wang MT, Honn KV, Nie D. Cyclooxygenases, prostanoids, and tumor progression. Cancer Metastasis Rev 2007;26:525-534.
- Wang X, Pankratz VS, Fredericksen Z, Tarrell R, Karaus M, McGuffog L, Pharaoh PD, Ponder BA, Dunning AM, Peock S, Cook M, Oliver C, Frost D, EMBRACE, Sinilnikova OM, Stoppa-Lyonnet D, Mazoyer S, Houdayer C, GEMO, Hogervorst FB, Hooning MJ, Ligtenberg MJ, HEBON, Spurdle A, Chenevix-Trench G, kConFab, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Domchek SM, Nathanson KL, Rebbeck TR, Singer CF, GschwantlRE-Kaulich D, Dressler C, Fink A, Szabo CI, Zikan M, Foretova L, Claes K, Thomas G, Hoover RN, Hunter DJ, Chanock SJ, Easton DF, Antoniou AC, Couch FJ. Common variants associated with breast cancer in genome-wide association studies are modifiers of breast cancer risk in BRCA1 and BRCA2 mutation carriers. Hum Mol Genet 2010;19:2886-97.
- Wang Y, Holland JF, Bleiweiss IJ, Melana S, Liu X, Pelisson I, *et al*. Detection of mammary tumor virus env gene-like sequences in human breast cancer. Cancer Res 1995;55:5173-9.
- Watanabe O, Shimizu T, Imamura H, Kinoshita J, Utada Y, Okabe T, Kimura K, Hirano A, Yoshimatsu K, Aiba M, *et al*. Expression of cyclooxygenase-2 in malignant and benign breast tumors. Anticancer Res 2003;23:3215–21.
- Weinberg OK, Marquez-Garban DC, Fishbein MC, Goodg- lick L, Garban HJ, Dubinett SM, Pietras RJ. Aromatase inhibitors in human lung cancer therapy. Cancer Res 2005;65:11287-91.
- Welcsh PL, Owens KN, King MC. Insights into the functions of BRCA1 and BRCA2. Trends Genet 2000;16:69-74.
- West SC. Molecular views of recombination proteins and their control. Nat Rev Mol Cell Biol 2003;4:435-45.
- Weston A, Godbold JH. Polymorphisms of H-ras-1 and p53 in breast cancer and lung cancer: a meta-analysis. Environ Health Perspect 1997;105:919-26.
- Whelan J, McEntee MF. Nonsteroidal antiinflammatory drugs, prostaglandins, and APC-driven intestinal tumorigenesis. In: RE Harris, ed. COX-2 Blockade in Cancer Prevention and Therapy. Humana Press, Totowa, NJ, 2002: 117-145.
- Wiegmans AP, Al-Ejeh F, Chee N, Yap PY, Gorski JJ, Da Silva L, Bolderson E, Chenevix-Trench G, Anderson R, Simpson PT, Lakhani SR, Khanna KK. Rad51 supports triple negative breast cancer metastasis. Oncotarget 2014;5:3261-72.
- Willems PJ. Susceptibility genes in breast cancer: more is less? Clin Genet 2007;72:493-4.
- Willett WC, Rockhill B, Hankinson SE, *et al*. Nongenetic factors in the causation of breast cancer. In: Diseases of the Breast, 3rd, Harris JR, Lippman ME, Morrow M, Osborne CK (Eds), Lippincott, Williams and Wilkins, Philadeliphia 2004. p.223.

- Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 1999;18:7908-16.
- Wolf I, O'Kelly J, Rubinek T, Tong M, Nguyen A, Lin BT, Tai HH, Karlan BY, Koeffler HP. 15-hydroxyprostaglandin dehydrogenase is a tumor suppressor of human breast cancer. Cancer Res 2006;66:7818–23.
- Wolf I, Sadetzki S, Catane R, *et al*. Diabetes mellitus and breast cancer. Lancet Oncol 2005;6:103.
- Wong CS, Lim GH, Gao F, *et al*. Mammographic density and its interaction with other breast cancer risk factors in an Asian population. Br J Cancer 2011;104:871.
- Wooster R, Bignell G, Lancaster J, *et al*. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378:789-92.
- Wooster R, Neuhausen SL, Mangion J, *et al*. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994;265:2088-90.
- Wren B. Hormonal replacement therapy and breast cancer. Eur Menopause J 1995;2:13.
- Wu F, Stutzman A, Mo YY. Notch signaling and its role in breast cancer. Front Biosci 2007;12:4370-83.
- Wu JQ, Li YY, Ren JC, Zhao R, Zhou Y, Gao ES. Induced abortion and breast cancer: results from a population-based case control study in China. Asian Pac J Cancer Prev 2014;15:3635-40.
- Wu KK. Cyclooxygenase 2 induction: molecular mechanism and pathophysiologic roles. J Lab Clin Med 1996;128:242-5.
- Wulfing P, Diallo R, Muller C, Wulfing C, Poremba C, Heinecke A, Rody A, Greb RR, Bocker W, Kiesel L. Analysis of cyclooxygenase-2 expression in human breast cancer: high throughput tissue microarray analysis. J Cancer Res Clin Oncol 2003;129:375–382.
- Wysowski DK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. Am J Epidemiol 1987;125:791.
- Xia B, Dorsman JC, Ameziane N, *et al*. Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet 2007;39:159–61.
- Xiao W, Oefner P. Denaturing high-performance liquid chromatography: a review. Hum Mutat 2001;17:439-74.
- Xue W, Warshawsky D. Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 2005;206:73-93.
- Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med 2006;354: 270-82.
- Yager JD. Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 2000;(27):67-73.
- Yaghjyan L, Colditz GA, Collins LC, *et al*. Mammographic breast density and subsequent risk of breast cancer in postmenopausal women according to tumor characteristics. J Natl Cancer Inst 2011;103:1179.

- Yan M, Rerko RM, Platzer P, Dawson D, Willis J, Tong M, Lawrence E, Lutterbaugh J, Lu S, Willson JK, *et al*. . 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-beta-induced suppressor of human gastrointestinal cancers. Proc Natl Acad Sci USA 2004;101:17468–73.
- Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, Gaudet M, Schmidt MK, Broeks A, Cox A, Fasching PA, Hein R, Spurdle AB, Blows F, Driver K, Flesch-Janys D, Heinz J, Sinn P, Vrieling A, Heikkinen T, Aittomäki K, Heikkilä P, Blomqvist C, Lissowska J, Peplonska B, Chanock S, Figueroa J, Brinton L, Hall P, Czene K, Humphreys K, Darabi H, Liu J, Van 't Veer LJ, van Leeuwen FE, Andrulis IL, Glendon G, Knight JA, Mulligan AM, O'Malley FP, Weerasooriya N, John EM, Beckmann MW, Hartmann A, Weihbrecht SB, Wachter DL, Jud SM, Loehberg CR, Baglietto L, English DR, Giles GG, McLean CA, Severi G, Lambrechts D, Vandorpe T, Weltens C, Paridaens R, Smeets A, Neven P, Wildiers H, Wang X, Olson JE, Cafourek V, Fredericksen Z, Kosel M, Vachon C, Cramp HE, Connley D, Cross SS, Balasubramanian SP, Reed MW, Dörk T, Bremer M, Meyer A, Karstens JH, Ay A, Park-Simon TW, Hillemanns P, Arias Pérez JI, Menéndez Rodríguez P, Zamora P, Benítez J, Ko YD, Fischer HP, Hamann U, Pesch B, Brüning T, Justenhoven C, Brauch H, Eccles DM, Tapper WJ, Gerty SM, Sawyer EJ, Tomlinson IP, Jones A, Kerin M, Miller N, McInerney N, Anton-Culver H, Ziogas A, Shen CY, Hsiung CN, Wu PE, Yang SL, Yu JC, Chen ST, Hsu GC, Haiman CA, Henderson BE, Le Marchand L, Kolonel LN, Lindblom A, Margolin S, Jakubowska A, Lubiński J, Huzarski T, Byrski T, Górski B, Gronwald J, Hooning MJ, Hollestelle A, van den Ouweland AM, Jager A, Kriege M, Tilanus-Linthorst MM, Collée M, Wang-Gohrke S, Pylkäs K, Jukkola-Vuorinen A, Mononen K, Grip M, Hirvikoski P, Winqvist R, Mannermaa A, Kosma VM, Kauppinen J, Kataja V, Auvinen P, Soini Y, Sironen R, Bojesen SE, Ørsted DD, Kaur-Knudsen D, Flyger H, Nordestgaard BG, Holland H, Chenevix-Trench G, Manoukian S, Barile M, Radice P, Hankinson SE, Hunter DJ, Tamimi R, Sangrajrang S, Brennan P, McKay J, Odefrey F, Gaborieau V, Devilee P, Huijts PE, Tollenaar RA, Seynaeve C, Dite GS, Apicella C, Hopper JL, Hammet F, Tsimiklis H, Smith LD, Southey MC, Humphreys MK, Easton D, Pharoah P, Sherman ME, Garcia-Closas M. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. J Natl Cancer Inst 2011;103:250-63.
- Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World Journal of Clinical Oncology 2014;5:412-24.
- Yong H-Y, Hwang J-S, Son H, *et al*. Identification of H-Ras-Specific Motif for the Activation of Invasive Signaling Program in Human Breast Epithelial Cells. Neoplasia 2011;13:98-107.
- Yoshimura N, Sano H, Okamoto M, Akioka K, Ushigome H, Kadotani Y, Yoshimura R, Nobori S, Higuchi A, Ohmori Y, *et al*. Expression of cyclooxygenase-1 and -2 in human breast cancer. Surg Today 2003;33:805–811.
- Yoshinaka R, Shibata MA, Morimoto J, Tanigawa N, Otsuki Y. COX-2 inhibitor celecoxib suppresses tumor growth and lung metastasis of a murine mammary cancer. Anticancer Res 2006;26:4245-54.
- Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. Science 2003;302:639-42.
- Yue W, Santen RJ, Wang JP, Li Y, Verderame MF, Bocchin- fuso WP, Korach KS, Devanesan P, Todorovic R, Rogan EG, Cavalieri EL. Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. J Steroid Biochem Mol Biol 2003;86:477-486.

- Zeng L, Biernacka KM, Holly JM, *et al*. Hyperglycaemia confers resistance to chemotherapy on breast cancer cells: the role of fatty acid synthase. Endocr Relat Cancer. 2010;17:539–51.
- Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632.
- Zhang S, Phelan CM, Zhang P, Rousseau F, Ghadirian P, Robidoux A, Foulkes W, Hamel N, McCready D, Trudeau M, Lynch H, Horsman D, De Matsuda ML, Aziz Z, Gomes M, Costa MM, Liede A, Poll A, Sun P, Narod SA. Frequency of the CHEK2 1100delC mutation among women with breast cancer: an international study. Cancer Res 2008;68:2154-7.
- Zhang SM, Cook NR, Manson JE, Lee IM, Buring JE. Low-dose aspirin and breast cancer risk: results by tumour characteristics from a randomised trial. Br J Cancer 2008;98:989-91.
- Zhang SM, Lee IM, Manson JE, et al. Alcohol consumption and breast cancer risk in the Women's Health Study. Am J Epidemiol 2007;165:667.
- Zhang SM, Manson JE, Rexrode KM, *et al*. Use of oral conjugated estrogen alone and risk of breast cancer. Am J Epidemiol 2007;165:524.
- Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 2003;95:373.
- Zhang X, Smith-Warner SA, Collins LC, Rosner B, Willett WC, Hankinson SE. Use of aspirin, other nonsteroidal antiinflammatory drugs, and acetaminophen and postmenopausal breast cancer incidence. J Clin Oncol.2012;30:3468-77.
- Zhang XH, Huang DP, Guo GL, Chen GR, Zhang HX, Wan L, Chen SY. Coexpression of VEGF-C and COX-2 and its associa- tion with lymphangiogenesis in human breast cancer. BMC Cancer 2008;8:4.
- Zhang Y, Coogan PF, Palmer JR, Strom BL, Rosenberg L. Use of nonsteroidal antiinflammatory drugs and risk of breast cancer: the Case-Control Surveillance Study revisited. Am J Epidemiol 2005;162:165-70.
- Zhang Y, Kiel DP, Kreger BE, *et al*. Bone mass and the risk of breast cancer among postmenopausal women. N Engl J Med 1997;336:611.
- Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 1996;137:5739-42.
- Zhao Y, Usatyuk PV, Gorshkova IA, He D, Wang T, Moreno- Vinasco L, Geyh AS, Breysse PN, Samet JM, Spannhake EW, Garcia JG, Natarajan V. Regulation of COX-2 expression and IL-6 release by particulate matter in airway epithelial cells. Am J Respir Cell Mol Biol 2009;40:19-30.
- Zhao YS, Zhu S, Li XW, Wang F, Hu FL, Li DD, Zhang WC, Li X. Association between NSAIDs use and breast cancer risk: a systematic review and meta-analysis. Breast Cancer Res Treat 2009;117:141-50.
- Zheng Q, Ye J, Wu H, Yu Q, Cao J. Association between mitogen-activated protein kinase kinase kinase 1 polymorphisms and breast cancer susceptibility: a meta-analysis of 20 case-control studies. PLoS One 2014;9:e90771.

- Zheng T, Holford TR, Mayne ST, *et al*. Lactation and breast cancer risk: a case-control study in Connecticut. Br J Cancer 2001;84:1472.
- Zheng W, Long J, Gao YT, Li C, Zheng Y, Xiang YB, Wen W, Levy S, Deming SL, Haines JL, Gu K, Fair AM, Cai Q, Lu W, Shu XO. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet 2009;41:324-8.
- Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE. Epidemiologic studies of estrogen metabolism and breast cancer. Steroids. 2015;99:67-75.
- Zielinska HA, Bahl A, Holly JM, Perks CM. Epithelial-to-mesenchymal transition in breast cancer: a role for insulin-like growth factor I and insulin-like growth factor-binding protein 3? Breast Cancer 2015;7:9-19.
- Ziv E, Cauley J, Morin PA, Saiz R, Browner WS. Association between the T29-->C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures. JAMA 2001;285:2859-63.
- Ziv E, Tice J, Smith-Bindman R *et al*. Mammographic density and estrogen receptor status of breast cancer. Cancer Epidemiol Biomarkers Prev 2004; 13:2090.
- Zujewski J Selective estrogen receptor modulators (SERMs) and retinoids in breast cancer chemoprevention Envir Mol Mut 2002,39:264-70.

APPENDIX: PUBLICATION FROM THIS THESIS