APOE AND ALZHEIMER DISEASE: A MAJOR GENE WITH SEMI-DOMINANT INHERITANCE

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Since the initial report of an enrichment of the *APOE* 4 allele of the Apolipoprotein E (*APOE*) gene among Alzheimer disease (AD) patients (Saunders et al. 1993), the strength of the association between different *APOE* genotypes and the disease is reported as odds ratios (ORs). Taking as a basis the most frequent genotype (*APOE* 33), the odds ratios are estimated to be 3.2 for *APOE* 34 and 14.9 for *APOE* 44 whereas, the *APOE* 2 allele has a protective effect in Caucasian subjects (Farrer et al. , 1997). However, ORs, that are basically epidemiological measures, are of limited interest in medical practise. What the physician (and the carrier of an " at risk " genotype) want to know is not the magnitude of the increased risk conferred by this particular genotype with respect to the most frequent genotype in the population but the actual probability to develop the disease according to age and sex. To address this issue, we (Bickeboller et al. 1997) and others (Myers et al. 1996 , Seshadri et al. 1995) previously attempted to calculate genotype-dependent AD lifetime risks (LTRs); i.e. the risk to develop the disease between birth and some particular age t (Thompson and Weissman, 1981). The LTR of a given *APOE* genotype could also be seen as the penetrance at age t of AD among carriers of this *APOE* genotype or the probability that a randomly selected individual with this *APOE* genotype will develop AD by that age assuming that he does not die of another cause before that age (Satagopan et al. 2001).

However, due to the limited sample sizes available at that time, 95% confidence intervals (95% CI) were huge and precluded any reasonable estimate of LTRs especially in *APOE* 4 homozygotes. Taking advantage of the large case/control sample used in a recent European AD Genome wide association study (GWAS) (Lambert et al. 2009) and adding two novel case/control cohorts, we undertook a novel attempt to estimate these values.

All subjects included in the GWAS as well as 2,971 new Caucasian subjects (1398 controls and 1573 AD patients) originating from the west of France and the USA and ascertained according to the same criteria than the GWAS subjects were included in this study. Therefore,

a total of 7,531 cases and 10,132 controls from seven different sub-studies were available. Demographic characteristics of the samples are summarized in Table 1.

Individuals were stratified into 4 age categories (less than 60, between 60 and 69, between 70 and 79 and more than 80 years old) depending on either the age of onset of the disease for patients or the current age for controls. *APOE* genotype frequency estimates and their 95% CI are shown in Figure S1 for cases and controls in the different samples stratified by age (males and females were considered together as no frequency difference was found between gender). After stratification on age, only limited heterogeneity could be seen between samples and we thus decided to pool them all. Table 2 gives the ORs (taking *APOE* 33 as reference) obtained for the different *APOE* genotypes on the pooled data in the different age categories and over all age categories. The OR of *APOE* 44 in the whole sample (OR=14.49; 95%CI = [11.91; 17.64]) is consistent with previous estimates (Farrer et al. 1997) but varies significantly with age, ranging from 5.6 (95%CI = [3.17; 9.89]) when the onset is before the age of 60 years old to 35.07 (95%CI = [23.8; 51.68]) when the onset is between 60 and 69. The same if true for *APOE* 34 with ORs in the range between 2.09 (95%CI= [1.61; 2.71]) for patients with an onset before 60 years and 4.18 (95%CI=[3.59; 4.88]) for the patients with an onset between 70 and 79 years. For the other *APOE* genotypes, ORs are more homogeneous across age-groups and are estimated to be 2.64 [2.13; 3.27] for *APOE* 24 and 0.56 [0.49; 0.64] for *APOE* 22 and 23 considered together.

Age dependant penetrances of the different *APOE* genotypes were computed using a method similar to those described in Bickeboller et al. (1997) and Satagopan et al. (2001). The only difference is that in our computation we account for mortality rates over the different age and genotype categories (see Supplementary Data for a description of the method used).

The AD LTR estimates according to age, sex and *APOE* genotype are presented in Table 3.

At age 85, they reach 51 % (95%CI=[41;69]) and 56% (95%CI=[45;75]) for *APOE* 44 male and female carriers and 23% (95%CI=[22;25]) and 26% (95%CI=[24;28]) for *APOE* 34 male and female carriers, consistent with semi dominant inheritance of a moderately penetrant gene. Clearly these values indicate that the effect of *APOE* on Alzheimer disease is more similar to the one of major genes in mendelian diseases such as *BRCA1* in breast cancer than the one of low-risk common alleles identified by recent GWASs in complex diseases. For a comparison, the lifetime risk of breast cancer in *BRCA1* mutation carriers by age 70 is estimated to be around 57% (95% CI, 47% to 66%), so very similar to our estimated *APOE* 44 penetrances by age 85 (Chen and Parmigiani ; 2007).

The allelic frequency of the *APOE* 4 allele in human populations ranges from 0.09 to 0.19 (Farrer et al. 1997). These values are well above those found for other deleterious alleles responsible for mendelian diseases, even those involved in recessive disorders. This apparent paradox might be explained by the fact that the *APOE* 4 allele, that is probably the ancestral *APOE* allele in humans (Fullerton et al., 2000), exerts its deleterious effect mainly in elderly individuals. Its impact on the reproductive fitness of the carriers is probably very limited. However, its reduced frequency compared to the one of the *APOE* 3 allele, if not due to genetic drift, suggests that it might slightly reduce the fitness.

As discussed by Yang et al. (Yang et al. 2009), lifetime risk estimates suffer from uncertainties in the population incidence rates, genotype frequencies and effect sizes. By using a bootstrap method, we were able to account for these two latter uncertainties but since we had to rely on published records of incidence rates, we were not able to model uncertainties in incidence rates. We also had to account for the fact that mortality rates vary depending on *APOE* genotypes. In particular, the *APOE* 4 allele has been shown to increase cardiovascular mortality (Schachter et al. 1994) and a recent GWAS confirmed that the *APOE* locus plays a role in longevity as this locus stands up among the ones with the most extreme

allele frequencies differences in centenarians compared to younger controls (Sebastiani, et al. 2010). To account for mortality differences depending on *APOE* genotypes in LTRs computations, we built a new model that assumes that for a given *APOE* genotype, survival probabilities are proportional to the genotype frequency differences observed over the different age categories in the sample (see supplementary data).

In conclusion, we think that our results urge for a shift of category of the *APOE* gene from "risk factor" to "major gene". This shift is not a pure semantic exercise. It has profound implications for patients. In Caucasian populations roughly 2% of the population bears an *APOE* 44 genotype. Considering the major risk conferred by this genotype, it becomes necessary to target at a presymptomatic state these individuals , as well as *PSENs* or *APP* mutation carriers, in clinical trials aimed at developing novel preventive therapeutics.

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	Cases			Controls		
Study	**Number**	**Age Mean (Standard deviation)**	**Male:Female**	**Number**	**Age Mean (Standard deviation)**	**Male:Female**
SPAIN	755	75.31 (9.33)	0.54	849	76.05 (12.37)	0.53
FINLAND	586	71.38 (7.50)	0.47	663	69.14 (6.09)	0.67
FRANCE 1	2 025	73.66 (8.91)	0.53	5 328	73.83 (5.42)	0.63
ITALY	1 513	76.62 (8.72)	0.48	1 338	70.76 (11.83)	0.80
USA	934	72.86 (6.26)	0.48	866	73.85 (6.36)	0.68
FRANCE 2	639	63.99 (9.88)	0.60	532	66.13 (12.01)	0.89
BELGIUM	1 079	78.56 (8.10)	0.51	556	64.20 (15.29)	0.75
TOTAL	7 531	74.03 (9.27)	0.51	10 132	72.37 (9.06)	0.67

Table 1 Summary of the characteristics of the different samples included in this study

Age-group	Sample Size (Cases/Controls)	APOE 44	APOE 34	APOE 24	APOE 22+23
Less than 60	593 / 610	5.60 [3.17;9.89]	2.09 [1.61;2.71]	1.29 [0.61;2.71]	0.43 [0.24;0.77]
60 to 70	1511 / 2614	35.07 [23.8;51.68]	4.18 [3.59;4.88]	2.2 [1.32;3.68]	0.59 [0.43;0.82]
70 to 80	3244 / 5175	15.75 [11.93;20.8]	4.54 [4.09;5.05]	2.79 [2.05;3.8]	0.51 [0.41;0.63]
More than 80	2183 / 1733	7.87 [4.28;14.46]	3.16 [2.68;3.72]	3.77 [2.28;6.23]	0.59 [0.47;0.74]
All	**7531/10132**	**14.49 [11.91; 17.64]**	**3.63 [3.37; 3.90]**	**2.64 [2.13; 3.27]**	**0.56 [0.49; 0.64]**
p-value[a]		**1.05×10^{-7}**	**3.40×10^{-8}**	**0.11**	**0.64**

Table 2 Odds-Ratios [95% Confidence Interval] of the different *APOE* genotypes (using *APOE* 33 genotype as reference).

[a] P-value of the Woolf test of homogeneity of OR between the different age categories.

Age	Gender	APOE 44	APOE 34	APOE 24	APOE 33	APOE 22+23
65	**Male**	4.14 [1.48;10.19]	1.02 [0.83;1.27]	0.28 [0.09;0.81]	0.28 [0.25;0.32]	0.21 [0.13;0.34]
	Female	4.25 [1.52;10.46]	1.06 [0.85;1.31]	0.29 [0.09;0.84]	0.29 [0.26;0.32]	0.22 [0.13;0.34]
75	**Male**	28.35 [21.21;43.63]	6.96 [6.35;7.62]	4.39 [3.14;6.80]	1.77 [1.69;1.86]	1.12 [0.92;1.38]
	Female	29.89 [22.39;45.96]	7.36 [6.72;8.06]	4.65 [3.32;7.19]	1.88 [1.79;1.97]	1.18 [0.97;1.47]
85	**Male**	50.92 [41.08;68.65]	23.22 [21.64;25.05]	19.99 [14.10;29.51]	7.76 [7.46;8.05]	4.87 [4.20;5.67]
	Female	56.03 [45.43;75.40]	26.09 [24.33;28.14]	22.55 [15.92;33.30]	8.76 [8.43;9.09]	5.51 [4.75;6.41]

Table 3 Penetrance estimates [95% Confidence Interval] (%) of the different *APOE* genotypes by different ages in males and females after accounting for *APOE* genotype differential effect on mortality using the model described in Supplementary Data.