FACULTAD DE MEDICINA
UNIVERSIDAD DE CANTABRIA

GRADO EN MEDICINA

TRABAJO FIN DE GRADO

Diagnóstico genético y medicina personalizada

Genetic diagnosis and personalized medicine

Autora: Dña. Delia Merayo López

Director: D. Gabriel Moncalián Montes

Santander, Junio 2018

Index

Resumen

El diagnóstico genético y la medicina personalizada como resultado del proceso de investigación y desarrollo en el campo de la genética desde el S. XIX, nos sitúa en una posición en la que a través de las nuevas técnicas de secuenciación masiva, somos capaces de conocer las secuencias específicas del genoma humano, de cada individuo, a fin de detectar patologías, riesgos de padecerlas y así diseñar un modelo de medicina dirigida a la predicción, la prevención y el tratamiento específico basado en las peculiaridades farmacogenómicas de cada paciente. No se concibe desarrollo de la genética sin el campo de la bioética cuyo desarrollo concomitante ha servido para proteger los derechos de los individuos. Con el aumento exponencial del rendimiento en las técnicas y la caída de los costes en los métodos de secuenciación de manera inversamente proporcional, el futuro de la medicina, seguirá pasando por la aplicación de la genética en la práctica clínica del día a día en nuestros hospitales.

Palabras clave: *Diagnóstico genético, medicina personalizada, bioética, genoma humano.*

Abstract

Genetic diagnosis and personalized medicine as a result of the process of research and development in the field of genetics since the 19th century, places us in a position in which, through next generation sequencing techniques, we are able to know the specific sequences of the human genome, of each individual, in order to detect pathologies, risks of suffering them. Thus, we could be able to design a model of medicine focused on the prediction, prevention and specific treatments based on the pharmacogenomic peculiarities of each patient. No development of genetics is conceived without the field of bioethics whose concomitant development has served to protect the rights of individuals. With the exponential increase of the performance in the techniques and the fall of the costs in the sequencing methods in an inversely proportional way, the future of the medicine, will be subordinated to the application of the genetics in the daily clinical practice in our hospitals.

Keywords: *Genetic diagnosis, personalized medicine, bioethics, human genome.*

1. Introduction

The aim of this introduction is to set a general background into the concept of genetic diagnosis and the personalized medicine which opens the pathway to delve into this work.

1.1 From mendelian times to the genome. The evolution of the molecular genetics

"The human genome underlies the fundamental unity of all members of the human family, as well as the recognition of their inherent dignity and diversity. In a symbolic sense, it is the heritage of humanity." (1)

From the very semantics of the word "health" (in the ancient Greek, origin) we find ourselves in the search for utopia, for success, for immortality. In this way, we find authors, works from all periods of history that have built a background on which we rely on today. With the development of genetics as a branch of biology from Gregor Johann Mendel in the nineteenth century and his experiments on inheritance through peas, he turned the study of heredity into a science. It has been long discussed about the real purpose of Mendel with his paper with a significant current of scientists supporting Monhagan's words, "The real objective of the work was the creation of a mathematically precise science of hybridization modeled upon the physical sciences" (41). What cannot be denied it is the impact in posterior minds through his discoveries regarding traits, hybrids and progeny.

Time passed and humanity witnessed the discovery of cellular division and the segregation patterns of chromosomes. Experiments on different species became to be performed with outstanding conclusions like the ones obtained by Morgan regarding the link between the behavior of chromosomes in meiosis and inheritance of genetic traits from the study *Drosophila melanogaster,* the vinegar fly.

It would be in the mid-twentieth century when "the molecule of life" came to light. The structure of double helix proposed by Watson and Crick and the knowledge provided on the mechanisms of information transfer in living matter would show the broad future options to keep developing this unexplored branch, the molecular genetics.

The scientific community began to realize the immediate application that this whole line of discoveries could have.

The key point of Mendel's discovery

1. Law of Development (2): A + 2Aa + a

from Mendel " a generally applicable law of the formation and development of hybrids".
"In the first and fourth terms germinal and pollen cells are alike; therefore the products of their association must be constant, namely A and a; in the second and third, however, a union of the two differing parental traits takes place again, therefore the forms arising from such fertil- izations are absolutely identical with the hybrid from which they derive. Thus, repeated hybridization takes place"(43). Therefore, the fertilization fractions are:

A/A + A/a + a/A + a/a = A + 2Aa + a

2. Developmental series(3):

1. Fertilization brings together pairs of contrasting traits to form the hybrid trait;
2. germ cells that carry only constant combinations of traits, in equal number, are then formed;
3. the germ cells unite randomly in fertilization;
4. the different classes of progeny make their appearance.

Starting in the early-sixties, Robert Guthrie introduced the screening of phenylketonuria (PKU) in newborns through the collection of heel stick blood for newborn babies being nowadays this screening test the standard for PKU.[4]

The beginning of neonatal screenings became a stepping stone for improved populations' health; the sooner the diagnosis was made and treatment began, the better the prognosis. This will be the main point of this document in itself, genetic diagnosis and personalized medicine for the individual.

1.2 Human Genome Project

The Human Genome Project (HGP from now on) was launched in 1990 with the goal of obtaining a highly accurate sequence of the vast majority of the euchromatic portion of the human genome. Since the discovery of the nucleotide sequence of bacteriophage phi X174 DNA in the 70's until the final outcome and completion of the HGP in 2003 an exponential race in knowledge took place, an amazing development of techniques and resources in which, with security, the competition of the public sector -the International Human Genome Consortium- and the private funds -with J.C. Venter leading Celera Genomics Corporation- led to lay a reference sequence of the human genome.

In this frame of continuous advance in researching, genomics and medicine the Human Genome Project is born in the Nineties. With an expected duration of 15 years, the primary objectives of the Project established by the NIH and the Department of Energy of USA, would be the ones that follow[5]:

1. Map and sequence the human genome
   - Build genetic and physical maps spanning the human genome.
   - Determine the sequence of the estimated 3 billion letters of human DNA, to 99.99% accuracy.
   - Chart variations in DNA spelling among human beings.
   - Map all the human genes.
   - Begin to label the functions of genes and other parts of the genome.

2. Map and sequence the genomes of model organisms

(The approximate number of base pairs in each species' genome is given in parentheses).
   - The bacterium *E. coli* (4.6 million)
   - The yeast *S. cerevisiae* (12 million)
   - The roundworm *C. elegans* (100 million)
   - The fruitfly *D. melanogaster* (180 million)
   - The mouse *M. musculus* (3 billion)

3. Collect and distribute data
   - Distribute genomic information and the tools for using it to the research community.
   - Release all sequence data that spans more than 2000 base pairs within 24 hours.
   - Create and run databases.
   - Develop software for large-scale DNA analysis.
   - Develop tools for comparing and interpreting genome information.
   - Share information with the wider public.

4. Study the ethical, legal and social implications of genetic research

5. Train researchers

6. Develop technologies
- Make large-scale sequencing faster and cheaper.
- Develop technology for finding sequence variations.
- Develop ways to study functions of genes on a genomic scale.

7. Transfer technology to the private sector

Online Education Kit. 1990: Launch of the Human Genome Project. National Human Genome Research Institute.

In June 2000 it was announced by USA president Bill Clinton that the majority of the human genome had been sequenced, years before the set deadline. Given the dispute between the private sector and the public one, in February 2001 the 90% of the sequence was published in Science and Nature respectively, providing a first overall view of the human genome; it would only take two more years for the final sequences to be published.

The draft sequences allowed systematic study of the human genome itself, including identification of genes, combinatorial architecture of proteins, regional differences in genome composition, distribution and history of transposable elements, distribution of polymorphism and relationship between genetic recombination and physical distance.

Whose genome was sequenced for the HGP?

The main internationally premise was to protect the identities of the volunteers who donated their biological samples for the cause. The samples where chosen from anonymous libraries 10 times bigger than the finally number chosen and with all identity labels previously removed.

"As the study of inherited variation between individuals, genetics might not immediately benefit from the sequence of a single genome. But even one genome would be immensely revealing to the science of deciphering the molecular blueprint of a species"[6].

The race for knowledge ended up in a politically correct dead heat between both sectors, which presented the assembled near-complete genome sequence with an error rate of only ~1 event per 100,000 bases and a final sequence containing 2.85 billion nucleotides with an approximately coverage of 99% of the euchromatic genome[7].

The HGP would lay the foundations of joint work in various areas, among specialists throughout the world in different fields towards a whole. All this harmonic structure was subjected to a deductive method based on the premise of the need for genome sequencing in order to improve our quality of life and health from the base. The project's new research strategies and experimental technologies have generated a steady stream of ever-larger and more complex genomic data sets that have poured into public databases and have transformed the study of virtually all life processes.[8]

1.3 Post-Genome Challenges

It was thought that the sequencing of the human genome would provide light directly to the complex functioning not only of human beings but our ancestry and species that had evolved simultaneously with the human race.

It was also thought that the mechanisms of health and illness would be revealed and an instant improve in our quality of life and life expectancy would be achieved. The answers were not only unsolved but a a broad new crossroad emerged in terms of new questions and possibilities.

From the basis that the most elementary of cellular functions - basic metabolism, transcription of DNA into RNA, translation of RNA into protein, DNA replication and the like - evolved just once and have stayed pretty well fixed since the evolution of single-cell yeast and bacteria [(9)] there are still huge unknowns on the table. We are far from knowing all genetic polymorphisms carried among the populations and therefore from identifying their association with diseases. In words of the International Human Genome Sequencing Consortium, there is an imperative need in the systematic identification of all functional elements in the human genome, including genes, proteins, regulatory controls and structure elements; requiring comparative analysis with many additional mammalian genomes and systematic application of diverse experimental techniques[(7)].

The pathways in this era are open to be followed. It will be a matter of time to keep fulfilling the established objectives. The set background of knowledges together with the international workforce and the current resources will lead to the development of the genetic diagnosis and the personalized medicine as we understand them; as strategies to prevent and treat eventual diseases and to make patients owners of the information regarding their state of health so that they would be the protagonists in the decision making process.

1.4 Molecular genetics and the concept of personalized medicine

Settled down in the previous lines we can understand molecular genetics and its historical development as a continuum with molecular medicine. Analyzing this discipline and what it implies, molecular medicine proposes to become a tool enabling to detect the individuals with potential risk to develop any illness even before the first symptoms may appear avoiding or delaying the corresponding manifestations, complications or aftermaths.

It will focus on the minimal differences of our genome which confer us determinate epidemiological risks, it will allow a medical practice much more specialized, a personalized medicine. More preventive, more predictive it will also make possible a better therapeutic approach through the knowledge provided about the “omics” (genomics, proteomics, metabolomics…).

Furthermore, thanks to the available information about celular pathways and the genes involved in diseases’ genesis we will be able to provide increasingly individualized treatments to selected targets, shortening treatment times, improving individual responses to drugs and avoiding undesirable adverse reactions.

It is time to put the theories in practice, we will next try to understand how.

2. Objectives

I. Through this work it is intended to review the concept of molecular genetics over time and its impact in personalized medicine until the present.

II. Through the state of art of the techniques up to the main applications in the day to day of the clinical and research practice we will see the part of truth and availability in the concept of "genetic diagnosis and personalized medicine".

III. Analysis of ethical and social implications of genetics, as well as the rights of human beings regarding health care and their privacy.

IV. Situation of the clinical specialty in comparison with other EU countries.

3. Methodology

3.1 Techniques

3.1.1. The Polymerase Chain Reaction

Developed in the eighties it supposed a landmark not only in medicine but in most fields of scientific research. It is a rapid DNA cloning method based on using the ability of DNA polymerase to synthesize a new strand of DNA complementary to the offered template strand, allowing quickly screening of multiple samples at a time.

It is a sensitive and robust cloning method widely used to quantitate both DNA and RNA, permitting the selective replication of one or more specific target DNA sequences within a heterogeneous collection of DNA sequences and enabling to increase vastly the number of copies (amplification)[10].

Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group (Figure 1), it needs a primer to which it can add the first nucleotide. This requirement makes it possible to delineate a specific region of template sequence that the researcher wants to amplify.

Through this oligonucleotide -complementary to the original DNA strand- acting as primer and another different one -in an antiparalele direction, as the proper DNA strands- at the end of the fragment of DNA chosen to amplify we will delimitate the desirable fragment.
As each synthesized piece serves as a basis for synthesizing others in the next cycle, the number of copies increases as follows:

> Partying from a single DNA molecule, in cycle 1 there will be 21= 2 fragments, in cycle 2 will be 22, that is, 4 newly synthesized fragments, and thus, with 35 cycles of PCR, 21 + 22 ... + 234 + 235 = 236 new fragments[11].

Thus, at the end of the Polymerase Chain Reaction (PCR), the specific sequence will be accumulated in billions of copies. The PCR depends on synthesizing oligonucleotide sequences to act as primers for new DNA synthesis at defined target sequences within the starting DNA leading to exponential amplification of the target DNA.

The process consists of a series of cycles of three successive reactions conducted at different temperatures repeated up to about 30-40 times in a standard PCR reaction[34] (Figure 2):

1. The starting DNA is heated to a temperature high enough to break the hydrogen bonds holding the two complementary DNA strands together. As a result, the double strands separate to give single-stranded DNA (denaturation). For human genomic DNA, the reaction mixture is generally heated to about 93-95ºC.

2. After cooling, the synthetic oligonucleotide primers are allowed to bind by base pairing to a complementary sequence on the single stranded DNA (annealing).

3. Next, in the presence of the four deoxynucleoside triphosphate dATP, dCTP, dGTP, dTTP, a purified DNA polymerase initiates the synthesis of new DNA strands that are complementary to the individual DNA strains of the target DNA segment.

The orientation of the primers is deliberately chosen so that the direction of synthesis of new DNA strands is toward the other primer-binding site. As a result, the newly synthesized strands can, in turn, serve as templates for new DNA synthesis causing a chain reaction with an exponential increase in product, as we can see in the figure below.

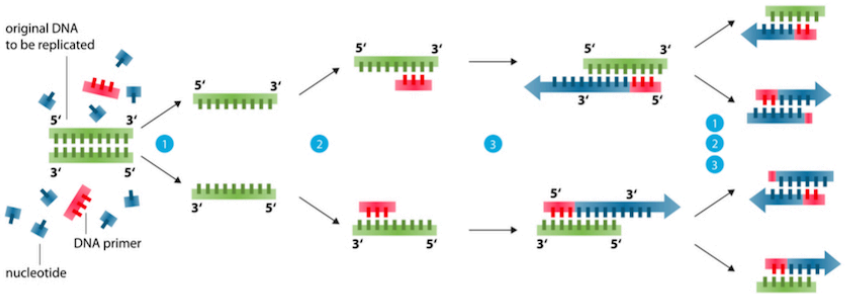

Figure 2. Diagram showing the main steps of the PCR: 1) Denaturation, 2) Annealing, 3) Elongation. Polymerase Chain Reaction: Steps, Types and Applications. Microbe Online *(12)*

The PCR reaction starts to generate copies of the target sequence exponentially. Only during the exponential phase of the PCR reaction is it possible to extrapolate back to determine the starting quantity of the target sequence contained in the sample. Because of inhibitors of the polymerase reaction found in the sample, reagent limitation, accumulation of pyrophosphate molecules, and self-annealing of the accumulating product, the PCR reaction eventually ceases to amplify target sequence at an exponential rate and a "plateau effect" occurs, making the end point quantification of PCR products unreliable (13).

The PCR has several applications depending on the element to study, and we can find several variations of the technique extending the original uses of the reaction (genetic fingerprinting-forensic application/paternity testing-, detection of mutation and investigation of genetic diseases, cloning genes…).

PCR is widely used in personalized medicine and genetic counselling for the amplification of the DNA region to study. Moreover, two remarkable processes performed through this procedure will be essential for the develop of the next generation sequencing technologies namely, the bridge PCR and the emulsion one. [See 3.1.3]

3.1.2. Sanger Method

One of the original methods for sequencing DNA, invented in 1977 by Frederick Sanger, is still widely used. This dideoxysequencing method generates a series of DNA fragments of identical sequence that are complementary to the DNA sequence of interest. These fragments differ in length from each other by one end base (Figure 3).

The complement of the gene of interest is copied into a collection of pieces, differing in the end bases, which are distinguished with a radioactive or fluorescent label. That is A, T, C, G are labeled with different fluorescent colors. Then the fragments are separated by size(14).

Once the areas of overlap are aligned, reading the labels end bases of the pieces in size order reveals the sequence of the complement, from which sequence of interest is derived.

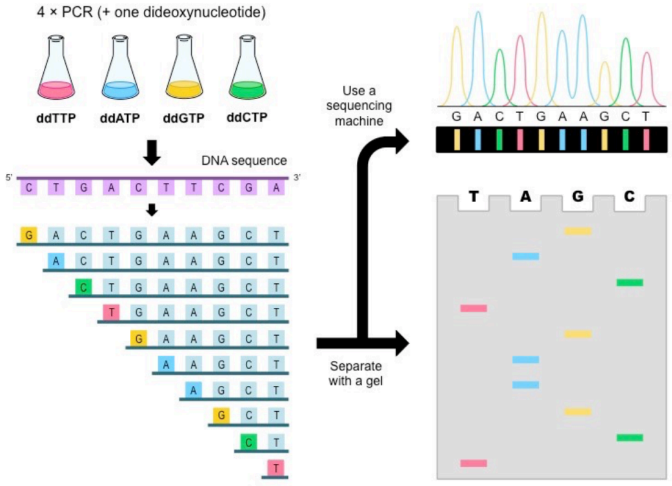

Figure 3. Process of gene sequencing through Sanger's method. Addition of a labeled ddNTP in each complementary fragment to stop the growing DNA strand. Subsequently, through electrophoresis, the fragments will be separated to proceed to its reading. Biology notes [15]

Why electrophoresis?

Besides being simple, cheap and easy to implement and optimize, its importance lies not only in its potential as a technique in itself when analyzing complex mixtures of macromolecules according to their size, but it is also the first step of many other more refined identification techniques such as the case in hand, gene sequencing[16].

Over the time automated DNA sequencing techniques appeared creating in one hand, a significant step forward in molecular biology and in the other, vast amounts of DNA sequence data at a higher rate that could be manually processed and interpreted. In this frame, the powerful software was created, such as Phred®, Phrap® or Consed®, in order to speed up the processing with improved accuracy.

As a tool of precision and in the personalized medicine itself, this method is used to detect single nucleotide changes, little insertions or deletions responsible for mutations in monogenic diseases. It was also used for diagnose of several kinds of cancer but as a greater number of genes and factors related to the genesis of tumoral processes have been discovered, this method has been substituted by modern techniques, the next generation sequencing[16]. Moreover, Sanger method is disadvantaged by relying on gel electrophoresis to fractionate newly synthesized DNA fragments. Not only does this make the method a laborious job, but more importantly it makes it difficult to sequence large numbers of DNA fragments at a time.

3.1.3. Next generation sequencing

Next-generation sequencing (NGS) is high-throughput, massively parallel sequencing. NGS is also referred to as second-generation sequencing technology, being the first generation composed by the original sequencing techniques of Sanger [17].

Essentially, all NGS platforms discussed below utilize the following steps: DNA (sequencing) library preparation, immobilization of library fragments on a solid support, amplification of the fragments, massively parallel sequencing of the fragments, and computer- aided assembly of the sequencea. In this process, each nucleotide base incorporated is detected by a "wash- and-scan" method; millions of reactions are imaged per run to achieve the massively parallel sequencing[18].

Compared to Sanger sequencing, advantages of the next-generation technologies alleviate the need for *in vivo* cloning by amplification of single molecules using either emulsion PCR (pyrosequencing) or bridge amplification on solid surface (Illumina). These based-in-PCR techniques previously mentioned, provide template amplification as a vehicle to conduce high throughput sequencing methods. They work as follows:

- **Emulsion PCR**

It is is based on compartmentalization of DNA fragments in minute water droplets/vesicles in a water-in-oil emulsion to a degree of dilution where there is only a single or a few template molecules per droplet. Ideally, each vesicle contains one sphere, one single-stranded template molecule, one of the primers bound to the sphere, and all other reagents necessary for the PCR reaction. Thus, every vesicle functions as an isolated PCR micro-reactor leading to generation of numerous copies of the bound templates facilitating signal detection from each one of the PicoTiter plate wells'[19] (Figure 4).

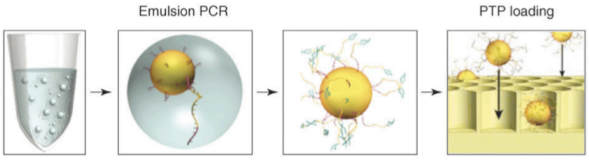

Figure 4. Steps in Emulsion PCR. Appreciate the contain of each droplet, a sphere used later as support for the DNA amplification to take place. Subsequently, the droplets will be loaded in the PicoTiter plate for the next step on the sequencing chain to happen.
Mardis ER. The impact of next-generation sequencing technology on genetics. Cell press (2007)[20]

It is important to employ a robust technique, as this one optimized to produce minimal PCR amplification bias and thereby providing output DNA most representative of the original sample.

- **Bridge PCR**

This reaction in contrast to the previous one, occurs on a solid surface, a flow cell.
The flow cell surface is coated with single stranded oligonucleotides that correspond to the sequences of the adapters ligated during the sample preparation stage. These single-stranded, ligated fragments are bound to the surface of the flow cell exposed to reagents for the polyermase-based amplification to occur. Priming takes place as the free end of a ligated fragment bounds to a complementary oligo on the surface so that, the repeated denaturation and extension results in localized amplification of single molecules in millions of unique locations across the physical surface (Figure 5).

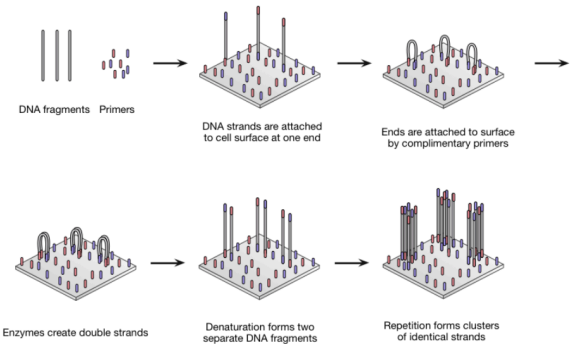

Figure 5. Steps in Bridge PCR. Amplification of single DNA strands through adaptators in a solid surface.
ATD Bio. Next generation sequencing[21].

Based on these two concepts, we will analyze the most commonly used high throughput sequencing technologies, pyrosequencing versus Illumina:

a) Pyrosequencing

Pyrosequencing is based on the sequencing by synthesis principle. When DNA polymerase elongates the DNA chain, pyrophosphates are released. Each released pyrophosphate triggers a series of reactions that generates a detectable quantum of light. Therefore, pyrosequencing enables real-time detection of the sequence of a gene. Thus, this technique is useful in the rapid detection of point mutations in the sequence and in SNP genotyping, including genotyping of microbes[18].

The DNA template that needs to be sequenced is first amplified by emulsion PCR as mentioned before. The amplicon (double-stranded amplified fragment) length is usually less than 200 bp for efficient pyrosequencing and the number of cycles in PCR for pyrosequencing is around 50 facing the 30-40 of the common PCR.

Pyrosequencing and its origin

The method was invented by a father seeking a way to quickly sequence his newborn son's genome[14]. What he did not know was that he would develop a technique able to sequence 20 million bases in about 4 and a half hours.

Pyrosequencing is conducted in 96-well plates, allowing hundreds of thousands of pyrosequencing reactions to be carried out in parallel, massively increasing the sequencing throughput.

During this process, the sequencing primer is first allowed to anneal with the DNA template in the presence of four enzymes—DNA polymerase, ATP sulfurylase, luciferase, and apyrase—and two substrates—adenosine 5′-phosphosulfate (APS) and luciferin—but without the deoxynucleotide triphosphates (dNTPs). Then, individual dNTPs are added to the reaction sequentially in a fixed order, which is programmed before the run.

If the added dNTP is complementary to the base in the template strand, it is incorporated by the DNA polymerase and a pyrophosphate (PPi) is released so that a emission of light is produced by the oxyluciferin (Figure 6). Conversely, if the injected dNTP is not complementary to the template base, no signal is produced.

The readout of this technique is called a pyrogram and it is used for comparing the one of the query DNA (sample) with that of the wild-type DNA (reference), being able to detect SNPs.

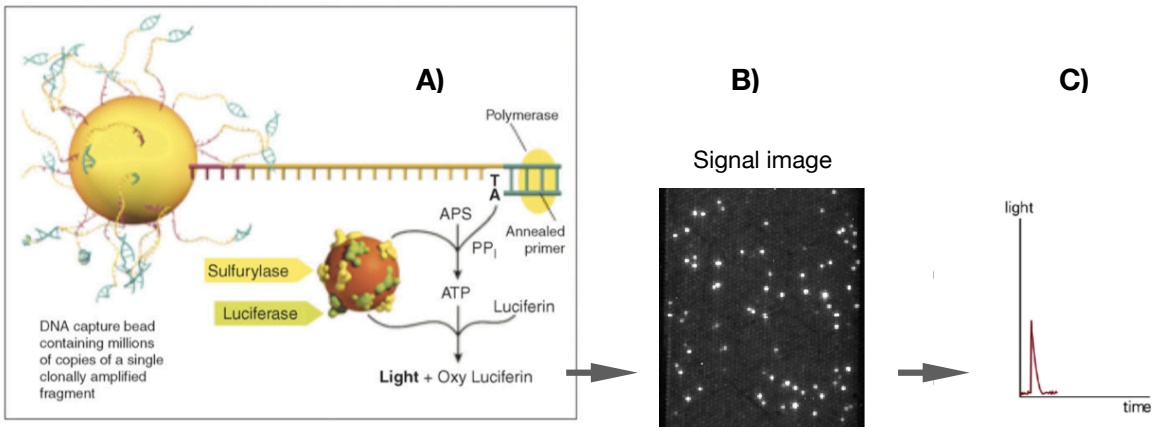

Figure 6. Diagram showing the steps in pyrosequencing reaction. A) The DNA polymerase incorporates dNTP complementary to the template strand. PPi released plus APS will produce ATP which will emit light in conjunction with luciferin. B) This light will be registered by the device camera (The fluorescence will be proportional to the amount of dNTP incorporated) C)The result is displayed in a pyrogram.
Mardis ER. The impact of next-generation sequencing technology on genetics. Cell press. Appell, M. Pharmacogenetic studies of thiopurines (2018).

Compared to the Sanger method, pyrosequencing is less costly and time consuming, although the applications of this technique do not completely overlap with those of the conventional method. The short-length (about 200 bp) sequences may be particularly suitable not only for screening specific SNPs but also for scoring entire haplotypes (groups of linked SNPs).

b) Illumina

The working principle is sequencing-by- synthesis chemistry as well. Input DNA is fragmented to generate <800 bp sequences which are ligated to the surface through specific adapters that have a single-base T overhanging. After denaturation, DNA fragments are immobilized at one end on a solid support, the flow cell.

Each flow cell is divided into eight separate lanes, each coated of covalently attached oligos complementary to the specific adapters. Each single- stranded fragment that is immobilized at one end on the surface creates a 'bridge' structure by hybridizing with its free end to the complementary adapter on the surface of the flow cell. The adapters on the surface also act as primers for the bridge PCR amplification [previously developed]. After several PCR cycles, about 1000 copies of single-stranded DNA fragments are created on the surface, forming a surface-bound colony (cluster)[22].

The flow cell is placed within the sequencer. During the process each cluster is supplied with DNA polymerase and four differentially labeled fluorescent nucleotides that have their 3′-OH chemically inactivated to ensure that only a single base is incorporated per cycle. Each base incorporation cycle is followed by an imaging step to identify the incorporated nucleotide at each cluster and by a chemical step that removes the fluorescent group and deblocks the 3′ end for the next base incorporation cycle to be repeated.

This series of steps are repeated “n” times to create a read length of “n” bases, as determined by user-defined instrument settings.

Afterwards, reads are aligned to a reference sequence with bioinformatics software being able to identify differences among the reference genome and the newly sequenced reads (Figure 7).

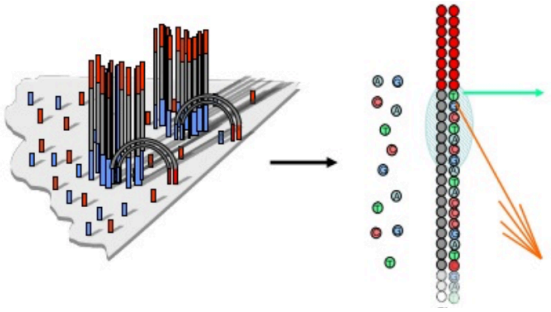

Figure 7. Illumina sequencing procedure. The different clusters will be labeled with fluorescent nucleotides which will be detected afterwards by the device camera.
Harvard Alumni. Illumina- shining light on your DNA[23].

As we found in pyrosequencing there will be a quality checking system to evaluate the data from each run, removing poor-quality sequences.

This masive-parallel sequencing method delivers quantitative measurements based on signal intensity detecting virtually all types of genomic DNA alterations, including single nucleotide variants, insertions and deletions, copy number changes, and chromosomal aberrations.

A particularly impressive increase in throughput has been achieved by Illumina -the enterprise that developed this technology- which currently offers the highest throughput per run and the lowest per-base cost[24].

Speaking of decreasing costs thanks to the aforementioned automation of techniques and the cheapening of procedures, the barrier of the $1000 genome corresponding to the original goal of the NHGRI funding program has already been broken; which means a 10 000-fold reduction in price relative to the cost of a human genome in 2004[77].

The following and well-known graphic (figure 8), perfectly illustrates this evolution in costs per genome far surpassing the predictions of Moore’s law.

Moore’s Law

(The number of transistors in a dense integrated circuit doubles about every two years).[26]

The direct consequence of Moore's law is that prices go down at the same time as benefits go up.

- understanding the benefits such as the availability of technology and its throughput increase.

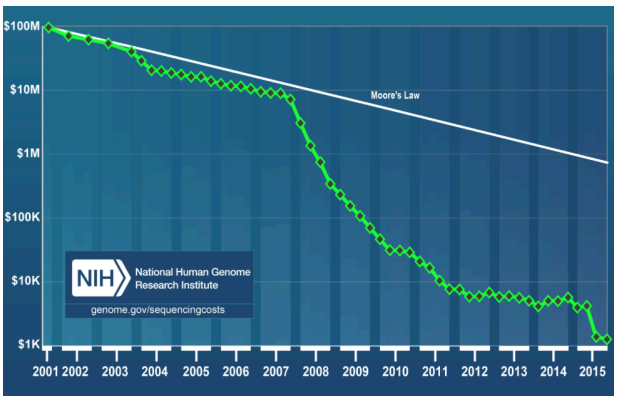

Figure 8. Exponential fall in costs of sequencing after the development of parallel mass sequencing methods. The fell even overpassed the prediction made by Moore. National Human Genome Research Institute. The Cost of Sequencing a Human Genome.

3.1.4. CGH array (Comparative Genomic Hybridization)

Microdeletions were long suspected to be the cause of some unexplained recurrent genetic syndromes or in many other individual cases in which a patient had a unique pattern of abnormalities, but until the development of comparative genomic hybridization (CGH) there had been no way to search systematically for them.

As a spin-off from the HGP, comprehensive sets of large purified DNA fragments (DNA clones) have been sequenced and ordered into linear maps corresponding to each chromosome. In array CGH the target DNA is a collection of such clones, that are deposited in defined geometric arrays of microscopic spots on a solid support (microarray). Through the array-CGH technology it is possible to give complete, overlapping coverage (tiling) of the human genome or to give high-resolution cover of a specific region of interest[10].

It uses competitive hybridization between DNA from a patient and from an unaffected control to identify chromosomal regions where there is a difference in copy number between the two. DNA samples from the patient and the control, labeled with different colored dyes, are mixed in equal amounts and hybridized to a sample from a normal individual into the microarray. Any difference in the number of copies of a particular DNA sequence in the unknown sample versus the reference DNA will affect the ratio of the two bound fluorescent dyes (figure 9).

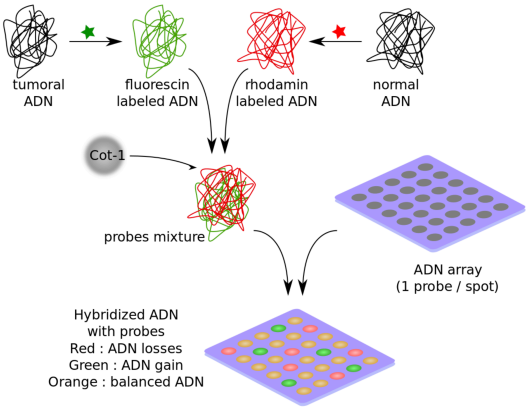

Figure 9. CGH array procedure of labelling two DNA samples for later hybridization. The different fluorescent signals will result in differences in copy numbers between a common DNA sample and the sample we want to test for alterations. Gbdivers (2008)

An example of the technique, CGH begins with the isolation of both genomic DNA from a tumor sample and genomic DNA from an individual who has a normal karyotype (control DNA). Next, the two genomes are differentially labeled and purified DNA is added to suppress the repetitive DNA sequences that are present in both genomes and therefore obtaining the desired hybridization signals with a minimum of background noise.

The differentially labeled genomes are then combined and hybridized to normal metaphase chromosomes.

The relative intensities of the green and red fluorochromes reflect the actual copy-number changes that have occurred in the tumor genome.
DNA losses and gains are indicated by a shift to red and green fluorescence, respectively.

Copy number variation (CNV) as the key of CGH-array techniques

This technique allows to detect chromosomal non-balanced abnormalities since the balanced ones such as, reciprocal translocations, mosaicisims, inversions or ring chromosomes, do not affect the copy number.

Array CGH applications are mainly directed at detecting genomic abnormalities in cancer. However, array CGH is also suitable for the analysis of DNA copy number aberrations that cause human genetic disorders[27].

Though not yet a widely employed for prenatal genetic diagnosis, the use of array CGH as a tool for preimplantation screening is becoming increasingly popular. It has the potential to detect CNVs and aneuploidy in eggs, sperm or embryos which may contribute to failure of the embryo to successfully implant, miscarriage or conditions such as trisomy 21 (Down syndrome)[28]. This makes array CGH a promising tool to reduce the incidence of life altering conditions and improve success rates of in-vitro fecundation attempts.

3.1.5. WGS vs WES

Notwithstanding the huge recent progress in genome sequencing, many current applications of massive parallel DNA sequencing are focused on target sequences that collectively constitute a small fraction of a genome. For example, screening for cancer gene susceptibility could involve sequencing all exons, and known regulatory elements for all known cancer genes to date. PCR amplification of what may be hundreds of sequence elements in each cancer gene is tedious and time-consuming. As an alternative, it is possible to use microarray hybridization as a tool to enrich for the desired sequences that are then submitted for high-throughput sequencing.

Another microarray-based application, genome-wide association studies (GWAS) have been a common approach for identifying disease associations across the whole genome. While GWAS microarrays can interrogate over four million markers per sample, the most comprehensive method of interrogating the 3.2 billion bases of the human genome is Whole Genome Sequencing (WGS). While WGS is commonly associated with sequencing human genomes, the scalable, flexible nature of the method makes it equally useful for sequencing any species, such as agriculturally important livestock, plant genomes, or disease- related microbial genomes. This broad utility was demonstrated during the recent E. coli outbreak in Europe in 2011[29], which prompted a rapid scientific response.

Using the latest NGS systems, researchers quickly sequenced the bacterial strain, enabling them to track the origins and transmission of the outbreak as well as identify genetic mutations conferring the increased virulence.

Genome-wide capture of essentially all human protein-coding exons, the human exome, for resequencing is an option too. Exome sequencing is a widely-used targeted sequencing method. The exome represents less than 2% of the human genome, but contains most of the known disease-causing variants, making whole-exome sequencing (WES) a cost- effective alternative to WGS [30]. With WES, the protein-coding portion of the genome is selectively captured and sequenced. It can efficiently identify variants across a wide range of applications, including population genetics, genetic disease, and cancer studies[31].

3.2 BigData

Medicine as one of the areas where technology has have one of the greatest impact in the last decades cannot elude the quiz of the moment, how to manage the huge flow of information regarding theory, patients' data, databases… and how to be able to keep it perfectly archived with easy availability of logarithms to integrate all these in each personal computer of each medical consultation with enough power to employ all the conglomerate of information in each individual patient with its individual characteristics. And of course, through intuitive software for the medical practitioners, not informatics and therefore without enough knowledge related to integration and relation of variables and complex data.

Widespread uptake of electronic health records plus the study of genomics -through sequencing, mapping and complex relational analysis between genes, variants and diseases- generates a vast amount of data; above all after the development of the HGP and the decreasing of sequencing prices from which the amount of stored information has been exponentially augmented.

How much does our genome cost?

The first sequenced human genome cost nearly $3 billion; the cost is currently less than $1,000 per genome, and $500 to sequence an exome, including reagent and the amortization of its machines[32].

The data must be clear, easy-to-manage and above all available not only to physicians and patients, but to scientists and clinical investigators if we are to continue to learn about the human genome. In the years to come, it will be tried to work out how we can handle it, store it, share it and definitely, make the best use of it[33, 34].

3.3 Epigenetics

The term epigenetics was introduced by C.H. Waddington in 1939 referring to "the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being"[35]. Epigenetics means 'above genetics' and it was originally conceived by Waddington himself to describe the existence of mechanisms of inheritance in addition to standard genetics[36]. Subsequently, epigenetics is redefined as hereditary changes in gene expression that are not due to alterations in DNA sequencing.

The essential difference between genetics and epigenetics is the reversibility of epigenetic modifications. This property gives the cells the dynamism necessary for a refined and meticulous control of gene expression. From an opposite point of view, it could suppose a disadvantage for human beings, such as the case of the inactivation of tumor suppressor genes, but the scientific community has been able to apply those mechanisms into the development of pharmacological therapies, such as the use of DNA demethylating therapies in patients with cancer, which showed proven efficacy in the re-expression of tumor suppressor genes for the treatment of leukemias and certain myelodysplastic syndromes[37].

Epigenetic mechanisms include DNA methylation, histone modifications, chromatin remodeling and microRNAs that act as regulatory molecules; conditioning the access to DNA of transcription factors, co-activators and co-repressors.

Chromatin conformation controls gene expression, both in the short term as cells respond to fluctuating metabolic and environmental conditions, an in the longer term as developmental programs unfold[10]. In this way, we will find that transcription requires an open chromatin conformation, whereas heterochromatin has a closed, tightly packed conformation and represses gene expression.

These different chromatin status, depending on the time scale, may be able to persist through DNA replication and mitosis, or even through meiosis. The best known heritable epigenetic changes are in X-inactivation and imprinting, which both result in mono-allelic gene expression.

There is currently little evidence for epigenetic changes a primary causes of human hereditary disease. Heritable DNA sequence variants can exert their phenotypic effect by triggering epigenetic changes, as in fragile X syndrome (figure 10), but heritable epigenetic changes without any underlying sequence change have not been unambiguously identified as causes of human hereditary disease.

Standard therapies are given to patients; however, few patients respond to these drugs, because of various molecular alterations in their cells, which may be partially due to genetic heterogeneity and epigenetic alterations. To realize the promise of personalized medicine, both genetic and epigenetic diagnostic testing will be required. [38]

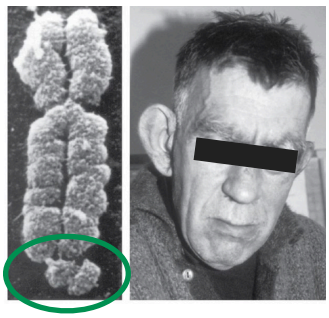

Figure 10.
Characteristic image of the affected segment of the X chromosome (green oval) and the phenotype of the patients.
Perini J. Malattie da mutation dinamiche.

Fragile X syndrome

Genetic condition that causes a range of developmental problems including learning disabilities and cognitive impairment. Usually, males are more severely affected by this disorder than females[99].

These individuals show a repeat expansion of triplet CGG, greater than 200 times. Methylation of this CGG triplet expansion will occur on the X chromosome, leading to the silencing of the FMR1 gene and a lack of its product, which conduce to the disease development.

This methylation of FMR1 in chromosome band Xq27.3 is believed to result in constriction of the X chromosome which appears 'fragile' under the microscope at that point, a phenomenon that gave the syndrome its name (figure 10).

3.4 Genetic counselling

It was 1947 when the geneticist Sheldon Reed coined the term “genetic counseling” for the advice he gave to physician colleagues on how to explain heredity to patens with genetic diseases[40].

It began in pediatrics and prenatal care and has become specialized so that counselors today focus on cancer, cardiac disease, neurology… The field has even infiltrated public policy as genetic testing has become widespread.

We will understand genetic counseling as a medical preventive tool and an educational process that aims to inform and advise patients and relatives at risk of a genetic condition about the nature of the disorder, the probability of developing it and the risk of passing it on to future generations.[41] Individuals can then make informed decisions about available testing, health management, treatment and family planning options.

Genetic counseling service may be useful at all stages of development, for instance babies undergoing screening, teenagers being tested for Thalassemia genes or assessing the genetic predisposition of adults as they enter mid-life to accommodate for lifestyle changes[42].

At the Marqués de Valdecilla University Hospital (HUMV, Santander, Spain), genetic counseling consultations are available in many services, both prenatal and postnatal. There are, for instance, monographic consultations in oncology guided mainly to family cancer syndromes where the scenario become a perfect inter conductor between genetics and its clinical application. Through the doctors in charge of these consultations, a detailed clinical record is performed in order to reflect each single a potentially relevant detail. Medical professionals as interlocutors play a main role face to the patients and explaining their singular conditions and needs of further investigations through additional complementary tests.

Our hospital in its portfolio of services also has strong presence in genetic counseling in pediatrics, in peripheral neuropathies (as in Charcot-Marie-Tooth disease), in otorhinolaryngology (above all referred to hearing loss) and emerging in cardiology regarding hypertrophic cardiomyopathy.

In genetic counseling for non mendelian conditions, risks are not derived from polygenic theory, they are empiric risks obtained through population surveys. This is fundamentally

different from the situation with Mendelian conditions, where the risks come from theory. The effect of family history is also quite different. Extracted from Strachan[10], I would like to reflect this through two very illustrative examples:

- If a couple have had a baby with cystic fibrosis, an autosomal recessive Mendelian condition, we can safely assume that they are both carriers. The risk of their next child being affected is 1 on 4. This remains true regardless of how many affected or normal children they have already produced; “chance has no memory”.

- If a couple have had a baby with neural tube defect, a complex non-Mendelian character, survey data suggest that the recurrence risk is about 2-4% in most populations. But if they have already had two affected babies, the survey data suggest the recurrence risk is substantially higher, often about 10%. It is not that having a second affected baby has caused their recurrence risk to increase, but it has enabled us to recognize them as a couple who always had been a particularly high risk. For multifactorial conditions, bad luck in the past is a predictor of bad luck in the future.

The Hardy-Weinberg distribution is very useful for predicting risks in genetic counseling as a model of the relationship between allele and genotype frequencies, both within and between generations, under assumptions of no mutation, no migration, no selection, random mating, and infinite population size. One generation of random mating is sufficient to establish a Hardy-Weinberg relationship between gene frequencies and genotype frequencies. In the absence of any disturbing factors, the frequencies will remain unchanged over the generations (Hardy-Weinberg equilibrium).

Random changes are often important in small populations. However, for a strongly disadvantageous disease allele, its frequency will basically depend on a balance between the rate at which fresh mutation are being created and the rate at which natural selection is removing them if deleterious[43].

Resource and technological advancements have the genetic counseling to play a major role in the care of many developed countries and presently its potential is being realized in the less developed countries. But this is still a burgeoning field; it is limited in developing countries, where the duties of counselors are still being fulfilled by other healthcare professionals without formal specialization, such as the current situation of Spain within an already formally organized European Union.

3.5 Pharmacogenetics

Pharmacogenetics is the science that study the actions and interactions among drugs in each individual based on their own genes. Thus, we can try to predict each person different answers’ to a same drug meanwhile, pharmacogenomics will involve the drug effects regarding the whole gene expression.

When looking to a drug’s prospectus we can read a list of side effects, which are usually reported in percentages, for example: "dizziness and headache can occur in 5% of the population". With the knowledge provided by pharmacogenetics, in some cases the previous sentence could be complemented with "to patients with an X polymorphism in the YY gene, this drug will cause dizziness and headaches". Therefore, for patients with this

polymorphism, it may be advisable to look for an alternative drug, with similar therapeutic effects and without the side effects[44].

Among the principal and wide applications of pharmacogenetics, I would like to remark the ones as follow due to the ability of this field for becoming a tool to practice a personalized medicine and for:

- Obtaining better therapeutic responses and fewer adverse or toxic effects.
- Shortening treatment periods and, in hospitalized patients, reducing hospital stays when giving an adequate medication to each patient (both in terms of dose and active ingredients).
- Reducing the number of hits and deaths due to adverse reactions to medications as well as interactions in polypharmacy, and avoiding the development of new microbial resistances intending to limit the impact of the existing ones.
- Reducing the economic and health costs of patients for ineffective medications.
- Improving the efficiency of clinical trials in the last phases prior to commercialization.

In November 2017, the Illumina chairman Jay Flatley was honored with the Lifetime Achievement Award at the annual Personalized Medicine Conference. During his speech he expressed awe at recent breakthroughs in therapeutics, noting twenty-five approved drugs are tailored to patient genetic profiles and “about 40% medicines in clinical trials could be classified as precision therapeutics. In oncology, the figure rises to 75%”[45].

It is time for us, as doctors, to realize that times are changing, genetics and pharmacogenetics is not just another tool but the essential one to lay the structure of an individualized medicine for the best achievements to be reached. It is not the future but the present.

RIGHT10K Protocol.
Mayo Clinic
(Right drug, Right dose, Right time: Using Genomic Data to Individualize Treatment)

99% of all patients studies have some kind of genetic variant affecting pharmacologic metabolism and therapeutic responses.[46]

According to cost/benefit criteria, there are currently some priority indications that are included in the sectorial technological foresight report, published by Genoma España Foundation in 2009[47], and are the following:

- Chronic diseases that require a long therapy.
- Therapies that require long periods before being able to evaluate the effectiveness of the treatment.
- Situations in which inappropriate therapies can have irreversible adverse consequences.
- Treatments associated with adverse events and, as a consequence, high potential morbidity.
- High cost treatments whose efficacy can be predicted by a pharmacogenetic test.

At present, drugs are adjusted in our hospitals based on sex, weight, age, known allergies, liver function and renal function. However, the genotype of each patient is not taken into account in the genetic variants that influence the outcome of each clinical case (response to treatments, evolution and resolution of cases, unexpected adverse reactions ...).

Through a brief review of the pharmacological metabolism, we will see the most susceptible points for the action of pharmacogenetics, both at the molecular level as well as the organs and systems.

3.5.1 Pharmacologic metabolism

In the pharmacological response intervenes, among other factors, enzymes responsible for metabolizing drugs (and/or endogenous substances involved in the process), drug transporting proteins (and/or endogenous substances, such as neurotransmitters, hormones...) and receptors or therapeutic targets in general.
All these factors are known to be proteins, whose synthesis is controlled by their specific genes and may present genetic variants that condition their pharmacokinetics/ pharmacodynamics, that is, their therapeutic efficacy, their side effects and their toxicity(44).

Most of the pharmacologically active molecules are lipophilic, which easily cross cell membranes and then are easily transported by lipoproteins but, if they are not transformed into polar molecules that can be eliminated, they can accumulate in the fatty tissue and produce harmful effects.

This process of transformation of molecules is called biotransformation being the liver the main organ in which it takes place. Biotransformation consists of 4 main phases (figure 11) that we will comment briefly to understand more specific aspects of pharmacological metabolism:

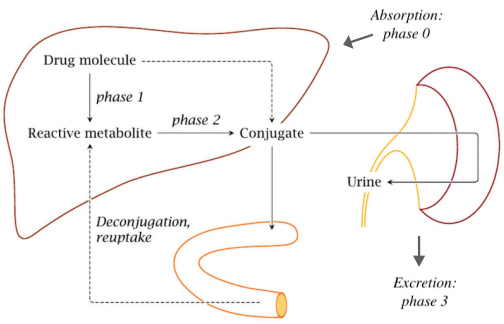

Figure 11. Diagram of the phases in pharmacologic metabolism, namely, absorption, first hepatic transformation of metabolites (CYP), hepatic detoxification (conjugation) and excretion of products.
Metabolism of drugs and xenobiotics. Univ. of Waterloo, CA

- **Phase 0.** Corresponding to the absorption process (passive or active) of the molecules and their distribution in organism. Given situations in which alterations occur in the barrier systems, we talk about processes related to the entrance of macromolecules, endotoxins, absorption deficits..

- **Phase I.** It consists in the transformation of the molecule into a more hydrophilic one through the release of a functional group via oxidation, reduction, isomerization or hydrolysis. It is mainly catalyzed by the MAO enzyme system dependent on cytochrome P450 (CYP).

- **Phase II.** In this phase, conjugation reactions will be carried out resulting in a molecule that is non-toxic and easily removable. The processes involved are: sulfation, glucuronidation, acetylation, methylation and conjugation with amino acids.

- **Phase III.** Final phase of the process that consists in the elimination of metabolites from the interior to the outside of the cell, through the plasma membrane. The tissues most involved in this process are those of the liver, kidneys, brain and placenta.

The variety of genes that codify for all the CYP superfamily (participants of the aforementioned phase I of biotransformation) can present silent SNPs if the change of base does not produce any change of amino acid, or in case of producing it, the modification of the post-translational structure does not confer any alteration in its activity. Conversely, the SNPs can produce a change of bases which modifies its capacity originating differences from normality.

These polymorphisms give rise to the different types of metabolizers, so that depending on the combination of individual alleles and the phenotype they express, we can find extensive metabolizers (if they maintain a level of activity similar to the normal threshold), slow or poor metabolizers, intermediate, fast and ultra-fast metabolizers, with the correspondent consequences that this entails in the metabolism of drugs.

For example, a person is poor metabolizer (PM) of a certain CYP. If the UNICAN drug is metabolized by this CYP with a SNP-PM, the enzymatic reaction will hardly take place, so that the substrate will accumulate. This can have consequences if the drug UNICAN itself is the metabolically active principle. Thus, it will be accumulated but not hydroxylated and so, difficult to be eliminated from the organism, giving place to an exaggerated effect of overdose. In these cases, it is possible to prescribe the medication but at much lower doses, being also a great help, the monitoring of plasma concentrations of the drug for the adjustment of the appropriate therapeutic window[44].

A more specific example, in a PM of CYP2C9, the anticoagulant effect of Warfarin, will not take place, not metabolizing and therefore accumulating the drug, being able to produce serious hemorrhages at usual doses.

Now, if the drug in question, it is a pro-drug that does not reach the active form until after the step of hydroxylation by CYP, the opposite situation will occur; there will be no pharmacological action and there will be side effects due to the accumulation of the drug, which as any chemical substance in high quantities, can give rise to adverse reactions.

In this case, we have the frequent example of people PM of CYP2D6 that, among other compounds, metabolizes most beta-blockers. Thus when administering Carvedilol to these patients, hypotensive effects will hardly occur but they will suffer from dizziness, headaches, etc.

Taking all this into account, it will be essential that the pharmaceutical companies, expressly indicate in the technical information of their medicines, through which CYP are they metabolized and if the active principle is the drug itself or if it is a prodrug.
It will be essential when decisions making for the implementation of certain treatments, adjustment of the posology and even thinking of drugs that at first could be assumed first line options being necessary to change them by others more suitable and beneficial for the patient and its situation.

All this information is collected in a hundred-and-five-page document regarding to susceptible prescription drugs and their routes of metabolism and it can be consulted online at the FDA website (genomic biomarkers table: https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm)[48].

3.5.2 In psychiatric diseases

The development of a predictive genetic profile of the efficacy, toxicity or adverse reaction of a drug is likely to be of immediate use for physicians to select a drug for the patient to not present risks of adverse effects, or to decide on a posology that guarantees an adequate treatment while minimizing complications.

Since the prevalence of mental illness is high in our society[49], it must be borne in mind that psychiatric disorders involve certain degrees of disability being essential the proper etiology handling in order to ameliorate people quality of life. In this sense, psychotropic drugs are widely and despite being effective compounds, they have a high response variability, as well as significant side effects, which together lead to therapeutic abandonment in numerous cases.

The main researching lines developed in the psychiatric pharmacogenetic field are mainly focused on the cytochrome P450 superfamily being the most relevant gene CYP2D6[44]:

- CYP2D6. It is the main route for psychotropic drugs used as numerous antipsychotics (Aripiprazole, Risperidone, Haloperidol…), tricyclic antidepressants (TCA)(Imipramine, Amitriptyline, Nortriptyline...) or anxiolytics such as Diazepam and selective inhibitors of serotonin reuptake (SSRI) such as Fluoxetine, Paroxetine, or Citalopram among others.
Its gene is very polymorphic, so most of the CYP2D6 alleles will not be defined by a single mutation or polymorphism, but by different combinations of them. The majority of the alleles confer alterations of the enzymatic activity endowing the individuals with null, reduced, normal and ultra-rapid metabolizations [see 3.5.1]. Clinically, PM and UM will be the most relevant insofar as they will serve to predict variations in plasma concentrations.
For example, TCAs have a narrow therapeutic window, so dose adequacy based on pharmacogenetics will be essential, whereas in the case of SSRIs whose therapeutic window is broader, CYP2D6 genotyping would be much more limited in cost-benefit terms.

3.5.3 In cardiovascular diseases

Pharmacogenetic studies related to cardiovascular diseases have largely focused on drugs that are already licensed and under generalized clinical use. This, undoubtedly, represents an additional obstacle because changing the accepted clinical practice, and therefore, professional behaviors, is difficult, since it often requires a higher level of evidence. Some of the greatest efforts regarding cardiovascular drugs were made towards anticoagulants and statins.

- **Anticoagulants.** The importance of the variations of SNPs involved in these subtypes of drugs, lies in the fact that they are widely used worldwide, prescribed in millions of patients, and because in people with certain SNPs they can have very serious side effects, from serious hemorrhages to thrombosis under the usual doses listed in the clinical protocols.

  - **Vitamin K antagonist drugs**: The anticoagulant action of coumarin derivatives is based on its ability to inhibit the VKORC1 gene, by a competitive mechanism, which prevents the stored form of the drug in the liver from being transformed into the active metabolite, VKH2. Warfarin (in America) and Acenocumarol (in Spain) are the most commonly prescribed coumarinic oral anticoagulants, they are indicated in patients with venous thromboembolism, atrial fibrillation and mechanical heart valves[50].

    In both drugs, the active compound is the molecule itself, that is, it will not need to be transformed into the liver, but when it is metabolized it will become inactive. Thus, the therapeutic action of this subgroup of anticoagulant drugs will be conditioned by two mechanisms:

A. In one hand, during phase I of hepatic detoxification, being the cytochrome more relevant for the inactivation of the compounds CYP2C9, so that according to their activity and their SNPs, different rates of metabolism will take place (as seen in chapter 3.5.1) in each individual. In case of being PM and since both drugs are active forms, at high doses there are high risks of hemorrhages.
B. On the other hand, we will see that the action of these drugs is not direct on coagulation factors, but indirect through their inhibition of the VKORC1 gene, and therefore, SNPs affecting the gene that codes for it will also modify the effectiveness of the drug in function that they confer an enzymatic capacity, EM, IM or PM.

We will have to handle coumarins with care due to their narrow therapeutic window, at low dosis there is no effect, at high dosis there are high risks of hemorrhagic events.

- **Platelet aggregation inhibitor drugs:** Generally speaking about Clopidogrel, it is a specific inhibitor of platelet aggregation that irreversibly and selectively inhibits the binding of ADP to its platelet receptor and, as a whole, prevents the effect of the glycoprotein complex involved in the process of platelet aggregation and clot formation[44].

  In this case, Clopidogrel is a pro-drug, whose metabolism through CYP2C19 will lead to the active metabolite with actions on coagulation. Thus, we will have the opposite situation to coumarinics; given the case of PM, after the administration of usual doses less conversion to the active drug will take place and consequently, a minor antiplatelet action and higher risk of thrombosis.

  Numerous cases of interactions between Clopidogrel and proton pump inhibitors (PPIs) have been described[52]. Both are metabolized mainly through CYP2C19 and, they can act as competitive inhibitors to each other. This fact highlights the need to be vigilant and periodically review the medications of our patients, both in polymedicated and in the case of without-prescription drug intake (as may be the case of Omeprazole that does not require medical prescription). Once more, this can lead to undesirable interactions and adverse reactions.

- **Statins.** The most commonly prescribed pharmacological class in the world, indicated for the primary and secondary prevention of cardiovascular disease. Its main mechanism of action is the reduction of LDL and cholesterol through competitive inhibition of the enzyme HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, a limiting enzyme for the synthesis of de novo cholesterol.

  Genetic variation in the lipid-lowering efficacy of statins has been widely investigated and more than 40 candidate genes have been described[51]. The result of these studies has identified as a relevant pharmacogenomic factor, the SLCO1B1 gene (Solute Carrier Organic Carrier Family Member 1B1), particularly the genetic variants rs4363657 and rs4149056, mainly related to muscle toxicity induced by statins.
  Being the frequency of myopathy and rhabdomyolysis induced by statins estimated at 1/1000 and 1/100,000, respectively, it has been reported that there is a strong association between these variants and the presence of severe myopathy, particularly demonstrated with Simvastatin[50].

Despite an increasing number of studies and associations established between pharmacological response and pharmacogenomics, most cardiovascular research of this type is in early stages, as researchers have found it difficult to identify and validate associations, which is largely due to the heterogeneity of patient populations and their phenotypes or to the difficulty of having adequate sample sizes.

3.5.4 In cancer

The oldest cancer treatment is surgery, which prevents invasiveness by removing the tumor. Two other common treatment approaches are radiation and chemotherapy, which kill all cells that divide rapidly. These treatments also affect healthy cells in the digestive tract, hair follicles, and bone marrow, causing side effects of nausea, hair loss, great fatigue and susceptibility to infection.
Several newer types of cancer drugs affect cancer cell characteristics or activities other than accelerated division rate, namely:

- Stimulate cells to regain specialized characteristics, such as drugs based on retinoid acid.
- Inhibit telomerase, which prevents cancer cells from elongating their telomerase and continually dividing.
- Induce apoptosis, which halts cell division.
- Inhibit angiogenesis, which orbs a cancer of its blood supply.

The approach to breast cancer illustrates how genetic and genomic information is refining management of these diseases.

The first targeting came with recognition that breast cancer cells have receptors for one or two hormones, estrogen and/or progesterone or neither receptor type. Some with estrogen receptor-positive tumors begin a several-year course of either a drug that blocks these receptors from receiving signals to divide, or a drug that inhibits the enzyme aromatase, which is required to produce estrogen. Determining the estrogen receptor status of a breast tumor is sub typing the disease by phenotype. With the discovery of single genes that cause cancer, diagnosis began to include genotyping (for example, a woman can have BRCA1 and/or HER2 breast cancer).

Increasingly, cancer diagnosis utilizes DNA microarrays that scan the genome for cancer-associated mutations as well as detect gene expression patterns. This approach of genetically characterizing tumor cells enables physicians to match a particular patient to the treatments most likely to work right from the start with the fewest side effects, and then to monitor response. DNA tests are also used to predict metastasis[14].

The benefits of personalized medicine in clinical oncology are undeniable. However, the search for an accurate result or the predictions of responses to a given therapy is a laborious task that requires a multidisciplinary effort that integrates molecular biologists, biochemists, oncologists, pathologists, pharmacologists... The validation of these markers and the methodological standardization of these therapies involve, in addition, diagnostic and pharmaceutical companies[44]. Generally speaking in medicine but particularly in this area the contribution of each of the parties is crucial to achieve the ultimate goal: to transcend clinical practice and to personalize the management of cancer patients.

3.6 Direct to consumer genetic testing

We have been talking about broad uses of genetic testing and their application on daily clinical practice but since the development of the human genome project itself and the premise of the human genome sequencing for lower budgets than 1,000 dollars, plenty enterprises have developed easy and intuitive tests for particular individuals. These tests allow knowing personal probabilities for the inheritance of determinate genetic traits (such as eye color, albinism, risk of developing diabetes...) and personal ancestry.

Typically, these tests require a blood sample, a swab from inside the cheek, or saliva. In direct to consumer (DTC) genetic tests (performed without requirement of physician's prescription), you collect the sample at home, the sample then is sent to a laboratory for analysis and in a variable period of time customers receive their results directly from a secure website or in a written report. The number of companies providing direct-to-consumer genetic testing is growing, along with the range of health conditions and traits covered by these tests, while associated costs are progressively decreasing.

In May 2013, Angelina Jolie wrote an editorial in the New York Times[53] about her personal crusade against cancer undergoing a preventive double mastectomy and a hysterectomy after taking a genetic test and checking her carrier status of mutations on BRCA1 gen, which augmented her risks to develop cancer at some point of her life. The editorial was one of the most viewed health related articles in the social media age[54]. Following her announcement, a study in The British Medical Journal using data from a large commercially insured US population[55], revealed a large spike in the number of BRCA testing requests after the editorial, highlighting the power of celebrity endorsements.

According to the Food and Drug Administration (FDA), which regulates the manufacturers of genetic tests, and the Centers for Disease Control and Prevention (CDC), some of these tests lack scientific validity, and others provide results that are meaningful only in the context of a full medical evaluation[56]. Due to the complexities of both the testing and the interpretation of the results, genetic tests should be conducted in registered laboratories that are certified to handle specimens, and the results may need to be interpreted by a doctor or trained counselor who understands the value of genetic testing for a particular situation. Moreover, positive results do not always indicate a clinical diagnosis but an increased risk for developing a disease or condition. Similarly, a negative result is not indicative of the absence of disease risk. These concepts can be difficult for consumers to understand without a physician or genetic counselor to fully explain them.

Other factors, including family background, environmental characteristics, current medications and medical history, also contribute to the likelihood of getting a particular disease. In most cases, genetic testing makes the most sense when it is part of a complete and addressed medical exam[56, 57].

Because there is currently little regulation of DTC genetic testing services, and the companies are forming at a rate that is faster than the patent and trademark office can handle the issuing of DNA patents, the US government has stepped in suggesting actions around the patent issue[43]:

- Ban the patenting of associations between DNA sequence variants and disease.
- Allow DNA to be patented only for use in a diagnostic test.
- Exempt physicians and researchers from litigation if they use patented DNA sequences.

- A broader action is the Genomic Research and Accessibility Act, which would ban patenting any DNA or its encoded proteins.

DNA patenting is evolving to embrace genome-wide applications: The DTC companies are finding themselves in an identity crisis. When these companies began to spring up a few years ago, they circumvented regulations on genetic tests for disease by claiming that they provided only information as an educational service. If the law disallow patent for use of DNA sequences in diagnostic tests, these companies would not be included because of how they identify themselves. But if the companies change their tune, claiming to offer tests for diseases so that they have access to patented sequences, they will be under scrutiny of the federal agencies that regulate genetic testing and products for health-related purposes.

We will have to wait a while to see how the whole picture evolves; of course due to the growth rate and the ease of access to these resources, regulations will be necessary so that the services provided are as reliable as possible for consumers. An increasingly sector of society who starts realizing its role in making decisions regarding health.

4. Discussion

It is undeniable that in any research practice in which living beings are handled and, above all, in the case that concerns us regarding human beings, comes into play the concept of bioethics.

As in the previous fields of this work we will see progress and updates over the time. After all, there must be a basis to protect the human rights, the dignity and privacy of all those who collaborate in the development of the scientific activity; to look after the ultimate beneficiaries of the personalized medicine, patients.

What dictionary says about bioethics:
"The study of ethical problems arising from biological research and its applications"[58].

4.1 Bioethics, a *continuum*

Throughout history we can find several milestones in the ambit of bioethics being essential the role of the Belmont Report[59] which promulgated guidelines for human experimentation in 1978, setting three main universal principles still maintained nowadays: respect for persons, beneficence and justice.

Bioethics in a continuous redefining situation, advanced meanwhile the concerns of the scientific community evolved, above all attending the greater possibilities and scope.
Thus, there was also a spot for bioethics in the HGP. Under the acronym of ELSI (Ethical, Legal and Social Implications) a research program was established in 1990 as a integral part of the HGP, receiving around a five percent of its budget, becoming the research project focused on ethical issues with the largest funds in history.

Although several of the topics regarding bioethics that were being addressed two decades ago (such as informed consent, privacy, issues related to the test results…) are still being

studied today, the topics are now being approached in more nuanced and methodologically complex ways. In addition, ELSI investigators have continually identified and addressed new issues (explorations of the human microbiome, genetic ancestry testing, and the growing impact of social media on the way genomic information is conceptualized and shared)[30]. The current priorities of ELSI project can be seen in the figure 12:

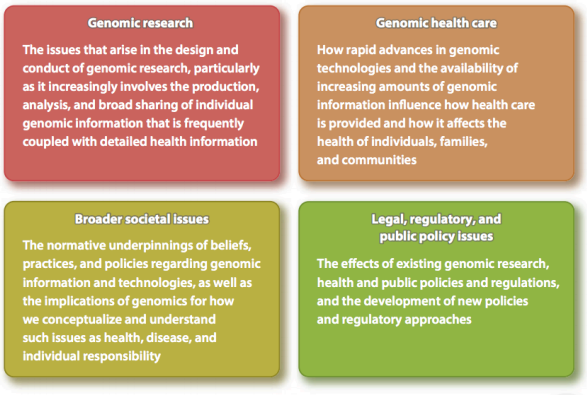

Figure 12. ELSI project priorities. *"The Ethical, Legal, and Social Implications Program of the National Human Genome Research Institute: reflections on an ongoing experiment"*. Annu Rev Genomics Hum Genet. 2014[60]

Talking about the situation of Spain in bioethics, we are ruled by the Biomedical Research Law (LIB), developed in Law 14/2007, which establish the scope of the research activity in our country as well as its limitations.

4.2 Legal regulations of biomedical research

According to the ruling law for the biomedical research in Spain (LIB)[61], we have to apply the concept of biomedical investigation to:

- Investigations related to human health that involve invasive procedures.
- The donation and use of oocytes, spermatozoa, pre-embryos, embryos and human fetuses (or their cells), tissues or organs for researching purposes and their possible clinical applications.
- The treatment of biological applications.
- The storage and movement of biological samples.
- Biobanks.

Until the approval of the LIB, we did not have a norm in our country that systematically proceeded with the regulation of the research activity in the clinical and basic areas. In the preamble of LIB, its defined that the main objective of this new law is to regulate biomedical research in order to preserve the full respect for human dignity and identity and therefore, for the people inherent rights'.

In the line of respect for the dignity and privacy of the human being, and focusing on the control over the use and destination of their personal information, it is interesting that LIB prohibits specifically the carrying out of genetic analysis by third interests from sectors such as labor or insurer. The practice of biomedical tests and further investigations will be absolutely restricted towards the sanitary scope and sub-estimated to the principles of information and consent.

Principle of consent: *"manifestation of the free and conscious will validly issued by a capable person, or by his authorized representative, preceded by appropriate information"*[61].

Principle of information: ***"****Everyone has the right to the information of their genetic and other personal data that has been obtained in the course of a biomedical investigation, according to the terms in which they expressed their will. The same right is recognized to the person who has contributed, for the indicated purpose the biological samples, or when it has been previously obtained other biological materials from them"*[61].

The LIB inspired by the precedent law 14/1986 (General Law of Health) and by the Official Law of Data Protection, is not complete in every of the needed spheres of bioethical concerns, so it applies in the cases not foreseen, the previous established regulations without changes. Thus, there will be concepts maintained from the old laws such as regarding to the figure of the patients as agents of the health system with decision-making capacity and with full control over their information; the consideration of genetic information as personal data is neither taking into account in the new regulations.

Also based on the principle of non discrimination -as usual in all international proclamations that have been pronounced on genetic data- the LIB supports the Universal Declaration on the Human Genome and Human Rights of UNESCO which states in its article number 6 that "No one shall be subjected to discrimination based on genetic characteristics that is intended to infringe or has the effect of infringing human rights, fundamental freedoms and human dignity"[1]. LIB also includes an entry referring that "neither may a person be discriminated because of his refusal to submit to a genetic analysis or to give his consent to participate in a biomedical research or to donate biological materials, particularly in relation to its corresponding medical-assistance benefit".

Regarding the limits of genetic analysis, the criteria of relevance, quality, equity and accessibility are established as rectors of the carrying out of genetic analyzes and once again towards the exclusive application to the healthcare field.

4.3 Limitations

The analysis carried out by Miguel Ángel Ramiro, from the Department of International Law, Ecclesiastical Law and Philosophy from the Carlos III University of Madrid, tries to show how the Law 14/2007 of Biomedical Research affects the performance of clinical trials with medicines in Spain among other issues due to ambiguities.[62]

The application of this law, in July 2007, directly affected the performance and evaluation of clinical trials with drugs through the creation of the Ethical Research Committees, changing the name of the concept but basically referring to the same pre-existing committees although without clearly defined competences. Something similar happened with the creation of the Bioethics Committee of Spain (articles 12 and 77 of Law 14/2007), an state consultative body, that would discuss individually the ethical and social implications of biomedicine and health sciences; the reality has been that it has not shown

significative visibility or activity, with what this means for the development of this field in our society.

It is undeniable that law has always lagged behind science, and a parallel evolution between scientific progress and the activity of the legislator is impossible; however, there is a growing awareness of the need to regulate the research activity and the peculiarities that genetic information presents in this area, in order to guarantee the rights and fundamental freedoms of the people involved in the development of the studies[44].

The LIB is a clear example of this awareness, when looking for a balance of interests, it will be essential to take into account the idea of necessary promotion of research without undermining the rights of patients; taking advantage of benefits obtained from scientific progress, but avoiding a distorted use of the results obtained and above all protecting the data processed in the development of the research activity.

Spanish postal service dedicates a print run to biomedical research in 2018[63]

There is still a long way for the field of bioethics to be defined as desirable in Spain. Thus, the task of those who are ahead of the advance will be to try to make the development of the research work as even as possible to the legislation, so that the faster the laws advance, the better we will be a able to apply the future that comes in terms of innovation, of genetics.

5. Conclussions

I think that everybody should take into account where we came from and this case is not an exception.

The world of genetics has had vertiginous growth in recent decades and that, in its totality is thanks to human potential. Pioneers for their times in fields like biology, chemistry, medicine that realized the weight of genetics, who directed their efforts to the description, to the development of models, technology and research lines that allowed us to know what we know nowadays. Another milestone, The Human Genome Project, the large capital investments, private companies and the pharmaceutical boom. In the end I think I could focus everything like the work of a painter: Devising in his mind what he wants to capture and starting to sketch a part of the canvas, then another, adding color and then details, getting the full picture.

I believe that all knowledge should have a great pursuit of utopia but also large doses of reality. I believe that the development and growth of biology along with genomics has been essential to serve and protect those who in the last instance are the beneficiaries of this herculean labor, people.

It is time to continue what others left us and keep working for the future path that others will take. I do not know how or where we will be within 10 or 20 years, but what can not be ignored is that future will be based on the application of genetics, personalized diagnosis, and therapies, in essence, on the development of medicine for the individual. Whoever refuses to see it, has not looked properly.

Being Spain the only country in the European Union that does not have officially the specialty of genetics in hospitals, we will have to make an exercise of introspection from medical schools to hospitals, and think about starting a new professional branch that will make our country maintain its quality level regarding public healthcare that citizens deserve.

“We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.”
T. S. Elliot [64]

I could not have finished this work with a sentence that would best summarize my ideas.

6. Bibliography

1. United Nations educational, scientific and cultural organization: *"Universal Declaration on the Human Genome and Human Rights".* Records of the General Conference 29th Session. Vol 1, Artic 1, Pag 42. (1997)
2. Monaghan FV, Corcos AF. The real objective of Mendel's paper. Biology and Philosophy 1990;5(3):267-292.
3. Sandler I. Development. Mendel's legacy to genetics. Genetics 2000 Jan; 154(1):7-11.
4. Gonzalez J, Monte S. Willis, MD: "Robert Guthrie, MD, PhD: Clinical Chemistry/Microbiology". Laboratory Medicine, Volume 40, Issue 12, 1 December 2009, Pages 748–749, https://doi.org/10.1309/LMD48N6BNZSXIPVH
5. The United States Human Genome Project. "*Understanding Our Genetic Inheritance. The First Five Years: Fiscal Years 1991-1995".* NIH. https://www.genome.gov/10001477/human-genome-projects-fiveyear-plan-19911995/
6. Chakravarti A. Single nucleotide polymorphisms:... to a future of genetic medicine. Nature 2001;409(6822):822.
7. International Human Genome Sequencing Consortium: "Finishing the euchromatic sequence of the human genome". *Nature* volume 431, pages 931–945 (21 October 2004) doi: 10.1038/nature03001
8. Collins FS, Green ED, Guttmacher AE *et Al.* on behalf of the US National Human Genome Research Institute: *"A Vision for the Future of Genomics Research"*. Nature, Vol. 422, No. 6934, April 24, 2003, p. 835-847.
9. Baltimore D. *"Our genome unveiled"*. Nature 2001 Feb 15;409(6822): 814-816.
10. Strachan T, Read AP. Human molecular genetics. An overview of mutation, polymorphism, and DNA repair 1999;2.
11. Asuar LE: "Guía practica sobre la técnica de PCR" Instituto Nacional de Ecología y Cambio Climático. Gobierno de México (online). Available at: http://www2.inecc.gob.mx/publicaciones2/libros/530/cap17.pdf
12. Acharya T: "Polymerase Chain Reaction (PCR): Steps, Types and Applications". Microbiome Online. 2016. (online). Available at: https://microbeonline.com/polymerase-chain-reaction-pcr-steps-types-applications/
13. NCBI. Polymerase Chain Reaction (PCR). 2017 (online). Available at: https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/
14. Ricki L. Human Genetics: Concepts and Application. WCB MGH 2005.
15. Karki G: *"Sanger's method of gene sequencing"*. Biology notes. (online). Available at: http://www.onlinebiologynotes.com/sangers-method-gene-sequencing/
16. Calabria I et al: "El nuevo reto en oncología: la secuenciación NGS y su aplicación a la medicina de precisión". Anales de pediatría Noviembre 2016 páginas 221-280 doi: 10.1016/j.anpedi.2016.05.006
17. Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics 2016;107(1):1-8.
18. Choudhuri S: "Bioinformatics for beginners". 2014 Pages 55–72 https://doi.org/10.1016/B978-0-12-410471-6.00003-7
19. Luthra R, Singh RR, Patel KP: *"Clinical applications of PCR. Methods in Molecular biology"*. Humana Press. 3rd edition (2016) DOI 10.1007/978-1-4939-3360-0
20. Mardis ER. The impact of next-generation sequencing technology on genetics. Cell press.Trends in Genetics Vol.24 No.3 doi:10.1016/j.tig.2007.12.007
21. ATD Bio. Next generation sequencing. (online). Available at: https://www.atdbio.com/content/58/Next-generation-sequencing
22. Zhou X, Ren L, Meng Q, Li Y, Yu Y, Yu J. The next-generation sequencing technology and application. Protein & cell 2010;1(6):520-536.

23. Harvard Alumni. “Illumina- shining light on your DNA”. Harvard Business School (online). Available at: https://rctom.hbs.org/submission/illumina-shining-a-light-on-your-dna/
24. Liu L, *et al.”Comparison of next-generation sequencing systems”* J. BioMed Research International 2012;2012.
25. NHGRI: “The Cost of Sequencing a Human Genome” 2016 (online) Available at: https://www.genome.gov/sequencingcosts/
26. Moore GE: "Cramming more components onto integrated circuits". Electronics Magazine. p. 4. (2011).
27. Oostlander AE, Meijer GA, Ylstra B: *“Microarray-based comparative genomic hybridization and its applications in human genetics”*. Clin Genet 66:488–495. (2004)
28. Evangelidou P, Alexandrou A, Moutafi M, et al: *“Implementation of High Resolution Whole Genome Array CGH in the Prenatal Clinical Setting: Advantages, Challenges, and Review of the Literature”.* BioMed Research International 2013.
29. Grad YH, Lipsitch M, Feldgarden M, et al. *Genomic epidemiology of the Escherichia coli O104:H4 outbreaks in Europe*,2011.PNAS.2012;109(8):3065–3070.
30. van Dijk EL, Auger H, Jaszczyszyn Y et al*: “Ten years of next-generation sequencing technology”,* Trends in Genetics, Volume 30, Issue 9, 2014, Pages 418-426. doi: 10.1016/j.tig.2014.07.001.
31. Illumina ® :*”An introduction to NGS technology”. 2017* (online). Available at: www.illumina.com/technology/next-generation-sequencing.html
32. Herper M : *“Illumina Promises To Sequence Human Genome For $100 -- But Not Quite Yet”.* Forbes Jan 2017
33. Heusel J, Richards N: *“Now we can cheaply sequence DNA, how do we store all that data?”* Wired Jan 2018.
34. Murdoch TB, Detsky AS: *“The inevitable application of Big Data to Health Care”* JAMA, April 3, 2013—Vol 309, No. 13 1351-1352
35. Waddintong CH. *“Preliminary notes on the development of the wings in normal and mutant strains of Drosophila”.* Proc Natl Acad Sci USA 1939;25:229-307
36. Bard, J. B. L. (2008). “*Waddington's legacy to developmental and theoretical biology”.* Biol. Theory 3, 188-197. doi:10.1162/biot.2008.3.3.188
37. Mack GS. “*Epigenetic cancer therapy makes headway”.* J Natl Cancer Inst 2006;98:1443-4
38. Rasool M, Malik A, Naseer M: *“The role of epigenetics in personalized medicine: challenges and opportunities”.* BMC Med Genomics. 2015; 8(Suppl 1): S5. doi: 10.1186/1755-8794-8-S1-S5
39. Genetics Home reference: Fragile X syndrome. NIH US National Library of Medicine (online). Available at: https://ghr.nlm.nih.gov/condition/fragile-x-syndrome
40. Wertz DC: “*Society and the Not-so-New Genetics: What Are We Afraid Of? Some Future Predictions from a Social Scientist”* J. Contemp. Health L. & Pol'y 299 (1997).
41. Nature: Genetic counseling. 2018 (online) Available at: https://www.nature.com/subjects/genetic-counselling
42. Genetic counseling services. Genomic resource centre WHO (online) Available at: http://www.who.int/genomics/professionals/counselling/en/
43. Griffiths. Modern genetic analysis. Integrating genes and genomes 2nd ed. W.H. Freeman and company. ISBN: 0-7167-4382-5 2002
44. Sabater J, Sabater G: Medicina personalizada posgenómica. Elsevier (2010). ISBN: 978-84-458-2025-4.
45. Davies K: *“Illumina’s Jay Flatley: In “Shock and Awe” at State of Personalized Medicine”.* Genetic Engineering and Biotechnology News. Nov 16, 2017.
46. Mayo Clinic Staff: *“Getting It RIGHT. 10,000 people help answer a basic individualized medicine question”.*

2017 (online). Available at: https://www.mayoclinic.org/giving-to-mayo-clinic/philanthropy-in-action/features-stories/getting-it-right
47. Galán OR, García MV, Toledano FG: *"Farmacogenómica: medicina personalizada y predictiva. Informe de prospectiva tecnológica sectorial."* Madrid. Genoma España; 2009
48. US Federal and Drug Administration: Table of Pharmacogenomic Biomarkers in Drug Labeling: https://www.fda.gov/downloads/Drugs/ScienceResearch/UCM578588.pdf 2017
49. Cuesta JP, Ruiz JS, Roca M, et Al: *"Salud mental y salud pública en España: vigilancia epidemiológica y prevención".* Psiquiatría Biológica, Vol 23, Issue 2, 2016, Pag 67-73, Psiquiatría Biológica doi: 10.1016/j.psiq.2016.03.001.
50. Quiñones LS, Roco A, Miranda C: "Farmacogenómica: Aplicaciones cardiovasculares", Revista Médica Clínica Las Condes, Vol 26, Issue 2, 2015 , pag 198-209
51. Verschuren J, Trompet S, Wessels J, *et Al*: *"A systematic review on pharmacogenetics in cardiovascular disease: is it ready for clinical application?"* Eur Heart J, 33 (2012), pp. 165-175 DOI: 10.1093/eurheartj/ehr239
52. Simon T, Verstuyft C, Mary-Krause M et Il: *"Genetic determinants of response to clopidogrel and cardiovascular events".* N Engl J Med. 2009 Jan 22;360(4):363-75. doi: 10.1056/NEJMoa0808227.
53. Jolie A: "*My medical choice"* TNYT. May 14th 2013.
54. PPI. genes, testing and the Angelina Jolie effect. http://www.advisortalk.ca/2018/03/14/genes-testing-and-the-angelina-jolie-effect/
55. Sunita D, Anupam JB: "Do celebrity endorsements matter? Observational study of BRCA gene testing and mastectomy rates after Angelina Jolie's New York Times editorial" *BMJ* 2016; 355 :i6357
56. USA Federal Trade Commission. Direct-to-Consumer Genetic Tests. 2018 (online) Available at: https://www.consumer.ftc.gov/articles/0166-direct-consumer-genetic-tests
57. American Medical Association. "Direct-to-consumer Genetic Testing". 2018 (online) Available at: https://www.ama-assn.org/content/direct-consumer-genetic-testing#Types%20of%20DTC%20Genetic%20Tests
58. Bioethics definition. Collins Dictionary. 2018 (online) Available at: https://www.collinsdictionary.com/dictionary/english/bioethics
59. The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. USA Gov: "The Belmont Report" April 1979 (online) Available at: https://www.hhs.gov/ohrp/sites/default/files/the-belmont-report-508c_FINAL.pdf
60. McEwen JE, Boyer JT, Sun KY, *et Al: "The ethical, legal, and social implications program of the National Human Genome Research Institute: reflections on an ongoing experiment".* Annual Review of Genomics and Human Genetics 2014;15.
61. Ley 14/2007, de 3 de julio, de Investigación Biomédica. (online) Available at: https://www.boe.es/buscar/doc.php?id=BOE-A-2007-12945
62. Avilés MAR: *"Impacto de la Ley 14/2007 de Investigación Biomédica en los ensayos clínicos"* UC3M (online). Available at: https://e-archivo.uc3m.es/bitstream/handle/10016/9119/impacto_ramiro_MC_2008.pdf?sequence=6
63. Sociedad Estatal de Correos y telégrafos. Emisión Ciencia. Investigación Biomédica en España. 2018 (online). Available at: https://www.correos.es/ss/Satellite/site/coleccion-1363199776559-galeria_sellos_filatelia/detalle_emision-sidioma=es_ES

64. TS Elliot quote (online). Available at: https://www.goodreads.com/author/quotes/18540.T_S_Eliot

Several images that have been obtained from third parties (and whose origin is specified in this section). Please consult the originators of those works for copyright restrictions that may apply to your intended reuse.

7. Acknowledgment

Thanks to the director of this work, Dr. Moncalián for his positivism, availability, knowledge and goodwill.

To each and every one of the people who have been part of this path.

To AJIEMCA and the International Federation of Medical Students Association (IFMSA-Spain) for helping me to know the priorities that every health professional should have.

To you who are reading this work, for the interest, because we have bet on the winning horse, medicine.

Thanks to the scientists and researchers of our country because despite the difficulties, they continue to contribute day by day. Genetics, the future, is in your hands.