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SPECIFIC WORK GUIDE ON "DIAGNOSIS AND GENE THERAPY"

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Introduction

All of us on some occasion have heard someone in our family say that we resemble one of our parents or the other. We have also heard that such a person had developed a disease that one of his or her parents had also suffered. We even speak about how we have inherited such and such a gesture from our parents. We are the way we are due to our genes, due to our DNA, inherited from generation to generation. Words like genetics, genome, DNA and hereditary diseases are now a part of our life.

The way in which this information was relayed from parents to children turned out to be an enigma until the middle of the past century. In 1944, O.T. Avary, C. McLeod and M. McCarty carried out a series of experimentswhich demonstrated that DNA (deoxyribonucleic acid) was the molecule which carries the genetic information and that this moleculewas relayed from one generation to the next. A few years later, in 1953, one of the most important milestones occurs in the history of genetics, when James Watson and Francis Crick published the three-dimensional structure of DNA; the double helix. In 1962, James Watson, Francis Crick and Maurice Wilkins received the Nobel Prize for this discovery, whichcompletely changed our way of understanding life in the end. After the discovery of the DNA structure, lots of discoveries followed about the mechanisms ofgene regulation, the genetic code was deciphered and lots of enzymes were identified which could be used to manipulate DNA. Among the scientists who contributed to reach these achievements is Spanish scientist Severo Ochoa, awarded the Nobel Prize in Medicine in 1959 for his discoveries about the mechanisms involved in the biological synthesis of ribonucleic and deoxyribonucleic acids.

Another of the most important advances was the discovery of DNA sequencing methods by Frederick Sanger and Walter Gilbert (Nobel Prize

in Chemistry in 1980), which made it possible to decipher thebase sequence ofA (adenine), C (cytosine), G (guanine) and T (thymidine), which formed the text of the DNA molecule. And a few years later, one of the essential discoveries in the decoding of the human genome was made;the discovery of the polymerase chain reaction (PCR), for which its inventor, K.B. Mullis, received the Nobel Prize inChemistry in 1993. This method revolutionized genetic research, enabling the development of all the technology which, years later at the end of the 20th century, would allow the first human genome sequencing.

A person's genome consists of 3 billion A,C, G and T nucleotides split up into 23 pairs of chromosomes. If we wrote the nucleotide sequence for any of us on paper, we would complete approximately 3,000volumes with 1,000 pages each, which gives an idea of the huge quantity of information stored in our genome. This DNA molecule contains genes, the elements which code for the proteins that take part in all the cellular phenomena. These genes define our physical appearance (skin color, eye color, length of the little finger and why we have two arms and not three), but also our susceptibilityto suffering illnesses. It is no surprise, then, that the scientific community set itself the object of decoding the complete human genome, with the hope of finding the answer to many human diseases.

The human genome project

The next step in understanding the molecular bases of life, and therefore of disease, was the decoding of thecomplete human genome sequence. In my opinion, human genome sequencingrepresents one of humanity's most important milestones, comparable to man reaching the Moon.

The debut of the Human Genome Project was held on June 26th, 2000 at the White House, wherethen-president Bill Clinton appeared, accompanied by scientists Francis Collins andCraig Venter, directors of the public and private consortiums. The results were published simultaneously (something which has happened very few times) in the top two scientific magazines, Nature and Science. The human genome project was completed in approximately 10 years with an estimated cost of over 2 billion euros. To that end, it was necessary to use thousands of automatic sequencers which worked day and night at specialized centers. These types of automatic sequencers are still being used nowadaysin genetic diagnosis units all over the world for the study of genes and specific DNA sequences.
The decoding of the complete human genome sequencelaid the foundation for modern medicine, enabling the discovery of new genes associated with disease.

New massive sequencing technologies

As we have commented previously, ten years and millions in investment were needed to decode the first human genome. However, in the past

few years new sequencing technologies have appeared called massive sequencing or ultrasequencing (ornext-generation sequencing) which enable a person's complete genome sequencing in a few days and at an extraordinarily low costif it is compared to the cost involved in sequencing the first human genome (the sequencing of all of a person's genes can currently cost about 1,000 euros). These new massive sequencing technologies are what has enabled the completion of international projects like the1000 Genomes Project, which consisted of the complete sequencing of the genome of 1000 individuals representative of the different human races, or the International Cancer Genome Consortium, ICGC,which is making it possible to know the most frequent mutations which characterize the most frequent tumorsand which will enable the development of newdrugs for the treatment of these diseases.

If students approach the websites of these international projects, they will quickly realize the huge amount of information which they are generating, and how this information is resulting in benefit of our health.

All of these projects produce an enormous quantity of information which has to be processed, stored and interpreted, which has led to the establishment of a new disciplinecalled bioinformaticsin charge of developing applications which are necessary for the analysis of this data and which has a huge professional future.

But ultimately, what is really important is how we transfer all of this information to clinical practice and the treatment of diseases. It's what has come to be called personalized medicine.

Personalized medicine

As its very name indicates, personalized medicineis none other than the application of treatments tailored to the patient, to get the best results with the least possible side effects. In order to select the best treatments, we must have the most detailed possible information about the diseasein each patient's specific context. In this field, genetic diagnosis will play a decisive role.

Let's imagine a patient who suffers from a malignant tumor. Studying the genome of the tumor will allow us to know the mutations which have appeared in his or her DNAand which genes are therefore altered. This information will be of great use to us to identify medication which acts specifically against the altered genes. But moreover, if we study the individual's genome, we will be able to know if the drugs that we use will be more or less toxic for the patient. The analysis of all this information will give us the most appropriate combination of medications for each disease in a particular person.

A field in which massive sequencing techniques have turned out to be crucial is in the field ofrare diseases. In Europe, a disease is considered

to be rare when it affects 1 in every 2000 people. There are calculated to be around 7,000rare diseases, so even though each disease affects a small number of individuals, as a whole, rare diseases affect about 7% of the world population. Many of these diseases are hard to diagnose and it is here where the genome sequencing of the affected people is playing a fundamental role.

Nowadays it is thought that there are still many rare diseases to be discovered. In order to tackle this problem, international consortiums have been established to identify these diseases (for example, the consortium called RD-Connect). Projects have also been established to respond to patients who don't find a definitive diagnosis of their disease (for example UDNI, Undiagnosed Diseases Network International; SpainUDP, the Spanish Program of Undiagnosed Rare Diseases). All of these international programs and consortiums are contributing to the increase of our knowledge about the molecular bases of the diseases, and what's more important, they are contributing to the improvement of the diagnosis of these patients and in certain cases identification of a cure for their disease.

One step further: gene therapy

A little over five decades ago, a series of visionary scientists hypothesized that genetic manipulation with exogenous DNA could be an effective treatment for hereditary human diseases. In this way, "gene therapy" would offer the chance to cure a disease with a unique treatment. Although the journey from the concept to its clinical application has been long andwinding, nowadays gene therapy offers treatments innumerous fields of medicine. Most of the treatments are still experimentaland are being tested by means ofclinical trials in humans (over 2,000 now completed or in progress). A few of these treatments have already been approved by regulatory agencies like the FDA in the United States or the EMA in Europe, and in the next few years the list of approved gene therapy medications is expected to increase substantially. Two examples of these already approved treatments are Strimvelis (Glaxo Smith Kline) for the treatment ofsevere combined immunodeficiency and Kymriah (Novartis) for the treatment ofacute lymphoblastic leukemia.

There are basically two types of gene therapy.The first one consists of the introduction of a normal copy of the altered gene directly into theindividual's somatic cells (this is calledgene therapy in vivo). In order to carry this out, viruses are mainly used which are injected directly into a particular organ such as the eyeand which are able to travel until the cells, enter them and deposit the normal gene there. This procedure, though apparently simple, is very complex and doesn't work in every situation. This strategy is being used to treat blood-clotting diseases, several types of blindness as well asneuromuscular diseases like Parkinson's andspinal muscular atrophy.

The other type of gene therapy consists of isolating the patient's cells, normally blood cells, which are genetically manipulated in the laboratory and afterwards transplanted back into the individual (this is known as gene therapy ex vivo). This procedure has turned out to be very promising for the treatment of many genetic diseases such assevere immunodeficiencies, sickle-cell anemia and beta-thalassemia.

One of the fields where gene therapy ex vivo is being the most successful is in the treatment of leukemia, a type of cancer which affects the blood cells. In this case, specific types of cells in the patient's immune system (T lymphocytes) are isolated and they are manipulated genetically in the laboratoryso that theyexpress a protein located in the cell membrane, which will recognize theleukemia cells and destroy them. These proteins from the cell membrane are known as Chimeric Antigen Receptors (CARs). This is a type of the so-called immunotherapy which isgetting really impressive results and which will very likely represent a turning point in the treatment of these kinds of blood cancers.

The CRISPR Revolution

One of the most exciting advances in the past few years has been the discovery of a new genetic manipulation tool which is being called on to revolutionize not just the world ofbiomedical research but also the world of medicine and gene therapyin particular. It is the CRISPR gene editing system. One of the most striking aspects of this technology is that it is based on the discovery of a primordial immune defense system present in bacteria to fight against the viruses which infect them. The first great discovery of this unique system in nature was carried out by Spanish scientist Francisco Mojica,who was studying the DNA of bacteria that live in the salt marshes of Santa Pola in the early '90s of the past century. This discovery laid the foundation so that scarcely two decades later, the mechanisms and basic elements defining this tool were established.

Basically, this gene editing system consists of an RNA molecule and an enzyme capable of cutting the DNA (this enzyme is called nuclease and the most famous one is the nuclease called Cas9). That simple.The impressive thing about the technology is that it is possible to lead thenuclease activity to nearly any area of the DNA just by changing the RNA sequence which serves as a guide. The system is so simple (and so cheap) that is has quickly been incorporated in research laboratories and it opens up endless opportunities for the guided manipulation of DNA …

Ethical implications

Each new breakthrough in science, especially inissues as sensitive as the one we are dealing with, always has implications in the field of ethics. After all, our genome is our property. Will we be ready for the changes which 21st-century genetics will have in our lives?

For example, what limits will we have to place on the generation of genetic information? Who will be able to have access to this information? Does greater knowledge of our predisposition to developing diseases modify our definition of disease? Is it ethical to use genetic information, for example, to choose the person we want to have children with, with the purpose of avoiding diseases in them? What limits will we impose on gene therapy? Will it be legal to modify genes in order to alter our physical appearance?

These and other questions, which we probably can't imagine yet, will appear as we continue to move forward in this exciting field of genetics and the application of gene therapy in the treatment of diseases.

Possible issues and topics for discussion

- The development of genetics has been exponential over the past few years, but its incorporation in clinics is unequal. Where do you think it is in Spain?
- Read up on a rare disease. Is there some type of genetic test for that disease? Is there some gene therapy to treat it?
- What ethical implications do you think the development of gene therapy has? Where would you draw the line?
- Think of some disease you would like to develop a cure for using gene therapy. Read up on the molecular mechanisms and genes involved and think about how you would carry out development of the therapy.
- Blade Runner, Gattaca, The Island. Have you seen any of these science-fiction films? What do you think of them?

Documentation for consultation (only as a guide)

Articles of scientific dissemination:

- The Medical Sleuth. Brendan Borrell. Scientific American Magazine: 305–November 2011
- Silent Mutations. J.V. Chamary and Laurence D. Hurst, Scientific American Magazine: 300 - June 2009
- Mapping the Cancer Genome. Francis S. Collins and Anna D. Barker,Scientific American Magazine: 296 - March 2007
- Genomes for All. George M. Church,Scientific American Magazine: 294 - January 2006
- A New Model for Defeating Cancer: CAR T Cells. Avery D. Posey, Carl H. June, Bruce L. Levine, Scientific American Magazine: 316 – March 2017
- What is a Gene? Scientific American Magazine – 2010. (Special Issue)
- New Genetics. Scientific American Magazine – 2004. (Special Issue)
- Special Genetics Edition: CRISPR. Scientific American Magazine.

Some features/blogs/articles published in the digital press (but always contrast the information and be critical)

- https://elpais.com/tag/terapia_genica/a
- https://elpais.com/tag/crispr/a/
- https://elpais.com/tag/genoma_humano/a/
- The year when gene therapy reached the markethttps://elpais.com/elpais/2017/12/21/ciencia/1513859506_288811.html
- Gene therapy avoids the disease which obliges receiving blood transfusions for life://elpais.com/elpais/2018/04/18/ciencia/1524073332_093232.html

International databases of the human genome, where you can find detailed molecular information about thepositioning of the genes in the genome, its structure, its function, etc.It is worth going through their websites to realize the huge amount of information which they store on the human genome (and other genomes):

- National Center for Biotechnology Information (United States): https://www.ncbi.nlm.nih.gov/genome/gdv/

- UCSC Genome Browser (University of California, United States):https://genome-euro.ucsc.edu/cgi-bin/hgGateway?redirect=manual&source=genome.ucsc.edu

- European Molecular Biology Laboratory – European Bioinformatics Institute – Wellcome Trust Sanger Institute: https://www.ensembl.org/index.html

Databases on diseases and genetic tests:

- OMIM, database on hereditary diseases: http://www.ncbi.nlm.nih.gov/omim
- Orphanet, website on rare diseases: http://www.orpha.net/consor/cgi-bin/index.php?lng=EN
- Eurogentest, website on genetic testing: http://www.eurogentest.org/

International projects to decipher the human genome and the genetic variability of the human species:

- Information about the Human Genome Project, where you will find numerous videos and documents about this international project: http://www.genome.gov/10001772

International projects to decipher cancer "genomes":

- CGAP (Cancer Genomic Anatomic Project): http://cgap.nci.nih.gov/cgap.html
- The International Cancer Genome Consortium http://icgc.org/

Genetic Diagnosis and Rare Disease Projects

- https://rd-connect.eu/
- http://www.udninternational.org/home
- http://portal-spainudp.pre.isciii.es/
- http://www.genome.gov/27544402

Have a look at these videos on CRISPR-style genetic manipulation technology

- The Spanish scientist who discovered the biological mechanism which is behind the CRISPR/Cas9 technology explains what it consists of https://youtu.be/BiIl9MBgsAA ; https://youtu.be/7pn-Nq8z7fo

Informative videos about CRISPR technology and its numerous possible applications in medicine

- https://youtu.be/ZoE-G7YPvZQ
- https://youtu.be/4YKFw2KZA5o
- https://youtu.be/Ft-160cAx38
- https://youtu.be/1BXYSGepx7Q (TED)
- https://youtu.be/TdBAHexVYzc (TED)