Clinical Genetics: Basic Concepts for Oncology Practice

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Abstract  Hereditary cancers are common in clinical oncology 5-10% of all diagnosed neoplasms are estimated to be hereditary cancer. Therefore, careful study of the family history, as well as knowledge of the basic principles of genetics is required to address this type of patient. The purpose of this article is to offer basic genetics concepts as a useful tool in oncology clinical practice with practical examples, as well as to justify why a genetics specialist should be part of the multidisciplinary team in patients with a suspected hereditary oncologic condition. (creativecommons.org/licenses/by-nc-nd/4.0/).

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INTRODUCTION: PRINCIPLES OF INHERITANCE

Each trait that Mendel identified in pea plants, such as shape, color, size, etc., corresponds to the expression of a different gene. The phenotype refers to characteristics we observe (morphological, physiological or biochemical), such as the eye color, whereas the genotype is the genetic constitution. The expression and interaction of the genotype with the environment enable the phenotype. As an example, we can mention eye color, which corresponds to the phenotype, whereas the genotype refers to the variants of each gene that influences the determination of this trait.

The term “gene” is defined as a specific DNA fragment or sequence that determines a trait; the gene is the fundamental unit of inheritance, and each gene’s different versions are known as “alleles” (alternative forms of a gene). For example, the EYCL3 gene is one of the genes that determine the eye color in humans, and there are two alleles for this gene: one for the blue (no brown) and one for the brown eye color.

All genes have a specific position within a chromosome, with this location being known as “locus”; the EYCL3 gene is at 15q11.2, which would translate as chromosome 15, long arm (q), band 11.2. This locus can be located by the allele for brown or blue (no brown) eye color.

The basic structure of a gene is made up by variable sized DNA regions that encode for specific amino acids in the protein called “exons”, while those regions in genes that do not encode for any amino acid are known as “introns” (Fig. 1).

HOMOZYGOTE AND HETEROZYGOTE: EQUAL AND DIFFERENT ALLELES

When both alleles in a locus are equal they are “homozygotes”, and when they are different from each other they are known as “heterozygotes”. In the eye color example, the brown color allele (B) dominates over the blue (no brown) color (b); this way, when a pair of different alleles (Bb) are present (heterozygocity), only the physical trait encoded by the dominant allele will be observed, i.e. brown eyes; conversely, blue-eyed (no brown-eyed) people would be homozygous for allele b (bb).

It should be noted that the above occurs as long as there is a specific combination of EYCL3 and other genes’ alleles, since eye color is a polygenic trait that is influenced by several genes that interact with the EYCL3 gene.

EXAMPLE APPLIED TO ONCOLOGY

Cytochrome CYP2D6

Cytochrome P450 is a family of enzymes in charge of metabolizing certain drugs. Among them, the enzyme product of the CYP2D6 gene metabolizes most parts of tamoxifen to convert it into its active metabolite, endoxifen. Most people normally metabolize tamoxifen; however, those who are homozygous for allele 4 (CYP2D6*4/CYP2D6*4) display the slow metabolizing phenotype and, therefore, tamoxifen metabolizes too slowly into endoxifen, which causes the treatment to be inefficacious. These patients are candidates to aromatase inhibitors such as letrozole rather than to tamoxifen. Heterozygotes with the CYP2D6*1/CYP2D6*4 genotype are expected to exhibit the intermediate metabolizer phenotype and to have reduced endoxifen levels, which implies clinical inefficacy. In the Mexican population, a frequency of 3.1% of CYP2D6*4 slow metabolizers has been found, as well as 20.7% of intermediate metabolizers, heterozygous for CYP2D6*4 (i.e. CYP2D6*1/CYP2D6*4).

The P450 cytochrome is an example of a gene that follows a Mendelian pattern of inheritance, where both alleles are expressed in the heterozygous status; i.e. it is a gene with a co-dominant pattern of inheritance. There are ongoing studies with large numbers of patients in order to determine if this pharmacogenetic study should be applied to all patients in routine clinical practice.

TYPES OF INHERITANCE

In the human species, chromosomes are classified as sexual chromosomes (X, Y) and autosomes (pair 1 to 22), which are identical between both genders; for this reason, an autosomal disease implies that the gene that causes the disease will be present in the autosomes, and both genders are usually clinically affected. In X-linked conditions, such as hemophilia, the gene that causes the disease is in sexual chromosome X and it affects predominantly males.

Diseases caused by a single gene in a specific locus are known as monogenic and follow a Mendelian inheritance pattern. An “autosomal dominant” disease occurs when a mutated allele produces the phenotype in spite of the other normal allele. On the other hand, “autosomal recessive” inheritance is observed when both chromosomes display the mutated allele; only homozygous individuals have the disease.

PENETRANCE AND EXPRESSIVITY

Penetrance is defined as the percentage of individuals with a particular genotype that expresses the expected phenotype. When a genotype fails to produce the expected phenotype, this is known as “incomplete penetrance”. Another related concept is “expressivity”, which is defined as the level of expression of a trait determined by a gene. “Variable expressivity” represents the expression spectrum of a specific genotype. Both incomplete penetrance and variable expressivity result from gene interaction effects and environmental effects, which can alter or partially or totally suppress the effect of a particular gene and, therefore, the sole presence of an altered gene does not warrant its expression. The phenotype is the result of a genotype that is expressed in a specific environment, i.e. each genotype can produce several phenotypes according to environmental conditions and interactions with other genes where the development occurs.

Example applied to oncology

The BRCA gene is a tumor-suppressor gene and is responsible for 60% of hereditary breast and ovarian cancer cases.
This gene takes care of DNA double-strand rupture repair, and is inherited in an autosomal dominant form, i.e. there is 50% risk that the offspring will inherit the mutant gene from affected parents. However, even if there is one affected allele, it will require the other allele to become damaged throughout life for cancer to be produced, which is known as the Knudson or two-hit theory (which will be explained later).

Penetrance of this gene is high but incomplete. Between 41 and 90% of people who inherit a mutation develop cancer sometime in their lifetime. Expressivity is variable: some people develop breast cancer, others ovarian cancer and, in a lesser proportion, cancer in other organs such as the pancreas and the peritoneum. In males, prostate cancer is more common, although they can also develop breast cancer. It should be noted that there will also be patients who will develop more than one malignancy throughout their lives, and there is risk for contralateral cancer (64%) and ovarian cancer (44%) after a first breast cancer.

The risk of cancer in BRCA-mutation carriers increases with age owing to incomplete penetrance and variable expressivity, but the development and onset of cancer cannot be accurately predicted.

**MUTATIONS AND POLYMORPHISMS**

Changes in genetic information that are not explained by preexisting genetic variability recombination are known as “mutations”. Mutations can be alterations in the number or structure of chromosomes or the result of changes in the DNA sequence, e.g. point mutations (changes or substitutions of one base for another).

Mutations are the source of evolution as they provide the raw materials for it to be carried out. Without mutations, genes would only exist in a single form and organisms would not be able to evolve and adapt to environmental changes.

Polymorphisms are genetic variants present in more than 1% of the population, while mutations are present in less than 1%. Variants of a single nucleotide are known as single nucleotide polymorphisms (SNP), and are distinguished from point mutations by their frequency in the population.

### Table 1. Main types of mutations

| Type of mutation          | Definition                                                                                     |
|---------------------------|------------------------------------------------------------------------------------------------|
| Substitution of one base  | Changes the nitrogenous base of a single nucleotide of the original DNA for another different one. Example: g.45576A>C, changes an adenine for a cytosine at genomic position 45576 of the gene. |
| Insertion                 | One or more nucleotides added in the original DNA sequence. Example: g.5756_5757insAGG, an insertion of the AGG nucleotides between positions 5756 and 5757, generating an increase of three bases in the gene sequence. |
| Deletion                  | Elimination of one or more nucleotides in the DNA sequence. Example: g.120_123del, a loss of nucleotides 120 to 123 of the gene sequence. |
| Frameshift                | Insertion or deletion of a number of nucleotides in the DNA (which is not divisible by three) that affects the original traduction of the protein. Example: c.288_289dupCC (p.Arg97Profs*23) nucleotide 288 and 289 are duplicated (CC) resulting in a change of amino acid arginine 97, which is the first amino acid that changes for proline creating a new reading frame ending at stop codon in position 23. |
| Expansion by trinucleotide repetition | Repeated sequence of three nucleotides that progressively increases in quantity, potentially altering the size of a gene. Example: c.53GCA[80], an increase in the number of repeated GCA (the 3 nucleotides repeated 80 times) in the encoding sequence of the gene. |
| Chromosomal               | Alters the number or structure of one or more chromosomes, thus affecting many genes that generate important phenotypical changes. Example: 47,XY, +21, trisomy 21, presence of an extra chromosome 21. |
| Genomic                   | Alters the haploid number of the species causing polyploidy. Example: 69,XXX, triploidy, presence of 3n, additional haploid complement. |
| Missense                  | Changes the wild type amino acid codon for another different one, thus altering the function of the protein. Example: c.4576A>C; p.Leu126Arg, the change of adenine for cytosine causes that wild type amino acid. |
| Nonsense                  | Changes the wild type amino acid codon for a stop codon, which causes premature termination of the protein, leaving it without function. Example: c.4576A>X; p.Leu126*, the change of adenine for another nucleotide causes the amino acid leucine to change for a protein termination codon. |
| Silent                    | Change an encoding codon for another synonym; therefore, the amino acid sequence of the protein is not altered. Example: c.4576A>C; p.Leu126-, in spite of the change of adenine for cytosine in the gene sequence, the new codon encodes for the same leucine amino acid. |

G: genomic sequence; p: protein sequence; c: coding DNA sequence.
Mutations can originate in any cell and at any stage of the cell cycle. If the mutation is produced in “somatic cells” (any cell but gametes), these cells produce identical daughter cells, which results in the mutation only being detectable in cells descending from the original cell where the mutation was initiated. If a mutation occurs in “germ cells” (in any of the gametes: ovule[s] or spermatozoid[s]), its effects are likely to be immediately expressed in the offspring; this mutation is present in all cells of an individual’s body. Table 1 illustrates the different types of mutations, as well as examples of nomenclature for each case.

**CANCER GENES**

A group of genes are the main regulators of cell cycle and cell division and death processes. Mutations in these genes are mainly responsible for the development of cancer.

In general, mutations occurring in genes that promote cell growth and division are dominant, since it only takes a single cell of the gene to be mutated for its effect to be produced; these genes are known as “oncogenes”. In healthy cells, oncogenes’ non-mutated versions are known as “proto-oncogenes”, which, when mutated, turn into oncogenes and contribute to the development of cancer.

Some genes repress cell growth and division when inactivated by recessive mutations, stimulate cell division. This effect requires both gene copies to be mutated in order to block cell division inhibition; these genes are known as “tumor-suppressor genes”. In this way, an organism can inherit a defective copy of the gene (heterozygous status) and not develop the disease since the other allele is wild-type (normal). However, heterozygous individuals are predisposed to develop cancer because wild-type allele inactivation is the only factor required to eliminate tumor-suppressor activity, a phenomenon known as “loss of heterozygocity” (LOH).

There is another group of genes called “DNA repair genes”, usually tumor suppressor genes, which correct errors during cell division. Mutations in these genes make cells prone to accumulate mutational errors associated with several types of cancer (colorectal, endometrial and gastric cancer).

**Examples applied to oncology**

An example of an oncogene is the KRAS gene; having a proto-oncogene like this one mutated confers poor prognosis for tumors. It has been studied in colon cancer and lung cancer, among others; if it is mutated, chemotherapy with cetuximab or panitumumab is particularly not recommended owing to the low response rate.

With regard to tumor-suppressor genes, there are the mismatch DNA-repair genes and MLH1, responsible for 90% of Lynch syndrome or hereditary non-polyposis colorectal cancer, an autosomal-dominant syndrome of variable expressivity and incomplete penetrance. Although the most common cancer is in the colon, there is also risk for cancer in other organs such as the stomach, small intestine, pancreas, kidney, endometrium, and ovary. It occurs at 50 years of age on average in the form of adenocarcinomas, predominantly in the proximal colon. Patients with this mutation have 52-82% risk of developing cancer throughout their lives. The MSH2 and MLH1 genes correct mismatch errors during DNA replication; when mutated, they promote other genes’ alteration.

**CANCER IS A GENETIC DISEASE**

Normal cells carry out several processes in response to internal and external (stimulating or inhibitory) signals: they grow, divide, mature, and die. In a cancer cell, one or more of these signals are interrupted, which makes the cell proliferate at an abnormally higher velocity owing to the loss of response to normal control mechanisms, in this way modifying the original shape and ultimately producing an abnormal cell mass.

Cancer arises due to DNA mutations that alter genes associated with cell-division regulation. Ionizing radiation and chemical substances are carcinogenic due to sometimes they break DNA at one or two of its strands, activating an oncogene. Viruses such as human papilloma virus produce cancer because they carry oncogenes that are able to confer immortality to the cells they infect. Other cancers are associated with chromosomal defects; in chronic myeloid leukemia there is a reciprocal translocation between chromosomes 9 and 22 that generates a hybrid BCR-ABL gene with oncogenic activity. Other cancers are of the hereditary type, owing to the inheritance of mutated tumor-suppressor genes.

Most tumors have dozens of point mutations in several genes, and some cancers may have more than 200 mutations, with 95% of them being point mutations. However, many of these mutations are deemed to be “passenger mutations” and do not confer a selective growth advantage; whereas a few are “driver mutations”, i.e. they promote tumorigenesis. A typical tumor has 2-8 driver mutations that are related to signaling pathways that regulate cell destiny, survival, and genomic maintenance processes.

**Example applied to oncology**

Retinoblastoma is an autosomal-dominant disease caused by an alteration in tumor-suppressor gene RB1. Knudson’s Hypothesis or double-hit theory explains the genetic basis of this cancer. Knudson proposed that retinoblastoma is caused by two different genetic defects and both have to occur for cancer to develop. In cases where the retinoblastoma
is unilateral, a single cell suffers two successive mutations. In cases of bilateral retinoblastoma both eyes are prone to cancer development, Knudson proposed that the patient inherits one of the two mutations (one hit) in all cells of the both eyes, second mutation in one eye-cell (two hits) is required to cancer development. In cases of unilateral retinoblastoma a single cell suffers two successive mutations (two hits). It should be noted that in these hereditary cases there is also risk for other type of cancers such as sarcoma, osteosarcoma, and melanoma.

CLASSIFICATION OF CANCER BASED ON ITS GENETIC ORIGIN

Sporadic cancer

Sporadic cancer results from acquired, sequential, and non-corrected accumulation of mutations in somatic cells. Activation of mutations in oncogenes and inactivation of tumor-suppressor genes are probably sporadic tumor’s first events, in addition to other independent mutations in at least 4-5 genes. Another characteristic is the age of presentation as expected for the type of cancer, generally it does not affect relatives, and usually there are aggregated environmental risk factors.

Hereditary cancer vs. hereditary cancer

Familial cancer occurs in several members of one family. However, it is not possible to recognize inheritance patterns, usually there are no mutations in hereditary cancer genes, and an increased risk for the development of cancer is known to exist for relatives.

Hereditary cancer

Hereditary cancer affects several members of a family and it is possible for inheritance patterns to be found. Generally, it occurs in people younger than expected for the type of cancer; these are more aggressive cancers and can be suspected based on clinical features. In hereditary cancers, it is important for germ mutations in one of the alleles of genes with high susceptibility for cancer to be identified. It is important to identify germine mutations in genes with high penetrance. For example, BRCA1, BRCA2, PALB2, CHEK2, TP53 and PTEN in hereditary breast and ovarian cancer.

GENETIC COUNSELING IN ONCOLOGY

Genetic counseling consists in offering information and guidance to the index case and his/her family about the role of genes and the possibility of their offspring inheriting the cancer risk, to explain international recommendations on currently available follow-up and even therapeutic and surgical strategies, as well as medical uncertainties and the available support to solve psychosocial problems. Finally, the benefits and limitations of molecular testing should be explained.

The risks for predisposition to cancer can vary among families due to the intervention of other modifier genes or the lifestyle.

Genetic counseling is an essential part of cancer diagnosis and diagnosis and should be carried out by a medical geneticist in Mexico. Adequate genetic counseling should be carried out before and after a molecular test is indicated.

CONCLUSIONS

Basic clinical genetics concepts were introduced, including topics about mutations, polymorphisms, nomenclature, notions, types of inheritance, as well as some interesting applications in the fields of pharmacogenetics, oncogenesis, hereditary cancers, and genetic counseling, which in future issues will be addressed in depth. Knowledge of the principles of genetics applied to clinical oncology will enable a comprehensive management of the patient and better understanding of genetic studies in the oncology practice.

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