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Genetic studies in the diagnosing of osteoporosis and other metabolic bone diseases

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Summary
The study of the genetic cause of a disorder depends on the clinical characteristics. If a specific genetic alteration is suspected, sequencing can focus on one gene, a panel of related genes, or the entire exome, depending on whether or not a gene is clearly suspected. The specific strategies depend on the condition under study and the diagnostic protocols implemented in each center. In the coming years, however, when costs tend to be lowered and analysis procedures are streamlined, sequencing of the entire exome will progressively replace gene panels. On the contrary, in case of suspicion of alterations of a broader chromosomal region, procedures that allow the detection of structural variants, in general some type of "array", are indicated. Interpretation of results, especially in the case of "variants of uncertain significance" often requires the judicious integration of genetic, bioinformatics and clinical data.

DNA AND MUTATIONS

The nucleus contains most of the genetic information, distributed throughout the approximately 3 billion nucleotides of human haploid DNA. The approximately 21,000 genes that encode the proteins necessary for the various organic functions are represented there, as well as an indeterminate number of genes that are transcribed into RNAs that do not encode proteins, but have regulatory functions[1].

Mitochondrial DNA is smaller, having about 16,000 nucleotides, with genes to encode 13 proteins and 24 non-coding RNAs (transfer and ribosomal)[2].

DNA changes that lead to disease can be classified according to various criteria, including:

According to frequency

- *Polymorphisms.* They are relatively frequent variations in the population, present in more than 1% of people. Its functional impact is generally limited. So individually they are not usually disease-causing. However, there are polymorphisms that are associated with a greater or lesser response to certain drugs. In addition, when several harmful polymorphisms occur in combination, or when adverse environmental circumstances coexist, they can determine the risk of suffering some processes, such as osteoporosis and other prevalent complex diseases, that have a "polygenic" inheritance.
- *Mutations.* They are rare. Depending on the specific region of DNA affected, they can induce very important changes in the activity of genes or the proteins encoded by them. Hence, a single mutation may be enough to cause disease. This is the usual case of classic, monogenic or Mendelian hereditary diseases.

According to the transmission

- *Inherited.* They are the genetic variants that are already present in the cells of the progenitors, including the germ cells, whether or not they have the disease. Logically, the parents carrying these mutations can transmit them to several descendants.

As is well known, depending on whether the presence of 1 or 2 mutated alleles is necessary for the disease to develop, they will show a dominant, recessive or codominant inheritance pattern. Careful analysis of the family tree will help to establish which of these patterns occurs, and whether the mutation affects the autosomes (in which case the disease appears equally in males and females), the sex chromosomes (in the most frequent cases, of recessive inheritance linked to the X chromosome, it preferentially affects males, but is transmitted by women) or mitochondrial DNA (both sexes are affected, but it is only transmitted through the maternal line).

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• *De novo*. These mutations are not generally present in the tissues of the parents, but appear during the formation of germ cells and persist in the embryo if they affect the ovum or sperm involved in fertilization. Other times, these mutations are not present in the germ cells of the parents, but appear during embryonic development.

According to the affected tissues

• *Germ mutations*. Whether inherited or de novo mutations, they are present in the germ cells of the parents, so that they are also present in all tissues of the embryo and later of the adult individual. They are, therefore, transmissible to the offspring

• *Postzygotic or somatic mutations*. They appear in some cells of the developing embryo, so that only tissues derived from these cells show the mutation. Depending on the moment of appearance, they can affect the whole of a certain tissue, or only a part of it, causing mosaicism, that is, the coexistence in the same individual of normal cells and cells with mutated DNA. Depending on whether or not the germ cells carry the mutation, they may be transmissible to the offspring.

According to the number of nucleotides involved

• *Point mutations*. The mutation affects only one nucleotide, in which the usual base changes for another.

• *Small group of nucleotides*. In these cases, they are usually insertions or deletions of a few nucleotides. Other times it affects repetitive regions, so that there is a change in the number of repeats of groups of 3-5 nucleotides.

• *Large regions*. Sometimes deletions or duplications affect large regions of DNA, which can include thousands or millions of nucleotides. Extreme cases are those in which an entire chromosome is lost (such as Turner syndrome, in which the Y chromosome is missing) or an extra extra chromosome is acquired (as in the trisomy 21 of Down syndrome).

It should be taken into account that during DNA replication some mutations often occur, most of which do not have a negative impact. Thus, an estimated 50 or so de novo mutations occur in each individual, of which 1 or 2 are located in the exome. The exome is the set of coding regions. Although it only represents 1% of the DNA (about 30Mb), it is assumed that it is the seat of more than 80% of the mutations that cause monogenic diseases. The accumulation over the generations, makes the exome of an average individual present some 20,000 point variants compared to the reference genome. Many more variants accumulate throughout the genome, including, on average in a given individual, about 1,000 "copy number variations" (extensive duplications and deletions), about 350,000 insertions and deletions of one or a few nucleotides, and more than 3 million mutations and point polymorphisms. This degree of variation, although it is a minimal percentage compared to the 3 x 10^9 nucleotides of the genome, represents a very important complexity when interpreting the results of genetic tests.

Sequencing and other genetic tests

There are different types of genetic tests, with different objectives and procedures. We will only comment on some of the most frequently used tests, highlighting the aspects of interest to the clinician in the field of skeletal alterations.

Karyotype

It is one of the classic procedures, especially useful when an abnormal number of chromosomes or other extensive structural abnormalities (large deletions or duplications) are suspected.

Genotyping using arrays

The matrices or "arrays" explore some specific nucleotides. In general, it is about 0.1-1 x 10^6 nucleotides distributed, either throughout the entire genome, or preferably in coding regions or more frequently causing disease. They can be useful for detecting some specific mutations, but clinically their main utility is the detection of variations in copy number and other extensive chromosomal abnormalities, as they have higher resolution than other techniques, such as karyotyping.

Sequencing

In this case, the sequence of a more or less large region of DNA is exhaustively investigated, so that the complete sequence of the region studied is obtained. Traditional sequencing methods (Sanger sequencing) were expensive and time consuming, so they could only be applied to relatively small regions. For this reason, today they have been largely displaced by the so-called "next generation sequencing" (NGS) massive sequencing techniques. These techniques can be used to sequence a gene, multiple genes, the entire exome, or even the entire genome.

Currently, the most common approach to the use of massive sequencing in the clinic is based on the following criteria:

- Although it is not the most frequent, in some disorders (for example, hemochromatosis) most of the patients present the same type of mutation. In these cases, as a first approximation, only one or a few nucleotides can be analyzed, by Sanger sequencing or by other simple and inexpensive procedures, such as allele-specific PCR.
- If there is a strong suspicion of which gene is involved, but there is allelic heterogeneity (that is, there are many mutations that can cause the disease), that gene can be sequenced (using classical or NGS procedures), or only the area coding (which includes the exons and the adjacent part of the introns) or the entire gene. An example of this situation is hypophosphatasia, whose characteristic biomarker is low levels of alkaline phosphatase, and which is due to mutations in the ALPL gene.
- If the clinical picture has characteristics that allow it to be grouped within a set of processes, but the specific gene is not easily predictable, a "panel" of genes can be sequenced using NGS that includes the genes usually involved in this type of process. Depending on the case, those panels may include just a few genes, or several hundred. It is the approach often used, for example, in case of suspected osteopetrosis.
- If the clinical picture is difficult to classify or it is a high genetic heterogeneity situation (for example, mental retardation) or if previous studies do not allow to find the genetic basis of the picture, the entire exome can be sequenced, the "clinical exome"(reduced version that includes only the genes that are known as associated with diseases, about 7,000) or even the complete genome.

Table 1. Analysis techniques frequently used in clinical genetics

Test	Typical resolution	Detected anomalies	Genes explored	Applicable without definite suspicion of the genetic cause
Karyotype	5-10 Mb	Aneuplodia, large structural alterations	All	Yes
FISH	50-2000 Kb	Structural anomalies	1 o more	No (yes)*
CGH	10-1000 Kb	Structural anomalies	All	Yes
Array SNP	50-400 Kb	Structural abnormalities, genotyping	All	Yes
MLPA	50 b	Structural variations of intermediate length	1/more	No
MS-MLPA	50 b	Structural variations of intermediate length, alterations in methylation	1/more	No
Genome (NGS)	1 b	Point mutations, short insertions/deletions, Variations in copy number*** (expensive and complex analysis)	All	Yes
Exome (NGS)	1 b	Point mutations, short insertions/deletions, Variations in copy number*** (does not detect mutations in non-coding regulatory regions)	~ 21000	Yes
Clinical exome (NGS)	1b	Point mutations, short insertions/deletions, Variations in copy number*** (limited to genes 30 years most commonly associated with disease)	~ 6000	Yes
Disease-oriented gene panels** (NGS)	1b	Point mutations, short insertions/deletions, (does not scan non-coding regulatory regions)	2-400	No
Unique genes (NGS or Sanger)	1 kb	Point mutations, short insertions/deletions	1	No
Genotyping (various techniques)	1 kb	Point mutations	1 nucleotide	No
Genotyping (multiplex techniques)	1 kb	Point mutations	2-50 nucleotides in 1 or more genes	No

B: base or nucleotide; NGS: next generation sequencing; *: procedures based on fluorescent probe in situ hybridization (FISH) are used in principle to explore a specific locus. However, several probes can be mixed to explore multiple regions and even "paint" all the chromosomes, thus being able to detect structural abnormalities with greater sensitivity than the conventional karyotype; **: sequence interpretation today is often a more expensive and laborious step than sequencing itself. This is why “virtual dashboards” analysis is sometimes carried out. It is also known as a "directed exome." That is, the entire exome is sequenced, but only the variants in the genes potentially related to the phenotype are then analyzed; ***: it is not the most sensitive technique, some may not be detected.

These procedures often pose difficulties to interpret the results, since many differences with the reference genome are usually detected), but in many cases it is difficult to establish whether they are pathogenic mutations or not. For this, a combination of bioinformatic strategies is used, together with the judicious interpretation of clinical data. Thus, for example, it is usually valued:

- The population frequency of the variants (the very frequent ones are probably not pathogenic).
- If these variants have been previously described as a cause of disease.
- If, in light of "in silico" prediction systems, the variants produce important functional changes in the protein sequence. However, at present many of the variants that are found are classified as of uncertain significance (VUS, “variants of unknown significance”).
- Whether the zygosity conforms to the inheritance pattern or not. Thus, if family history suggests an autosomal recessive pattern of inheritance, heterozygous mutations may not be pathogenic. However, in these cases we must not forget the possibility that it is a composite heterozygous individual (that is, that it has two different heterozygous mutations in each of the alleles of the gene).

It is also very useful to sequence the genome of the parents (which is sometimes known as the "exome trio", since it includes the two parents and the patient under study) to facilitate the interpretation of the variants found. If the parents are healthy, the variants of the patient that are present in any of them must not be pathogenic.

To confirm pathogenicity, it is often necessary to carry out a “segregation” study, that is, to analyze the suspected variant in other relatives, to check whether this mutation is present in patients and absent in healthy ones.

Detection of structural variants and other sequencing limitations

Mass sequencing techniques have been a true revolution in genetic studies. They make it possible to determine extensive DNA sequences in a short time and at a relatively low and decreasing cost. However, it must be taken into account that these techniques allow us to know the nucleotide sequence in the analyzed DNA, but they present some limitations:

1. Although there are algorithms that make it possible to determine if there are alterations in the number of copies of the analyzed regions, these procedures are not completely effective in detecting **structural variants**. Therefore, in case of suspicion, carrying out procedures that are more sensitive is recommended to detect these types of alterations. Among them it is worth highlighting:

   a. *Comparative genomic hybridization or differential hybridization arrays (CGH arrays)*. They carry probes distributed throughout the genome. The results obtained in the patient are compared with those obtained in a healthy subject. They usually have a resolution between 40 and 400 kilobases.

   b. *Arrays of SNPs*. They analyze nucleotides scattered throughout the genome and make it possible to determine whether or not there are two copies of each of them, thus detecting the deletions and duplications that may exist. It should be noted that these techniques do not detect some structural variants that do not involve changes in the number of copies, such as rearrangements and inversions.

   c. *Amplification of probes after multiple ligation (MLPA, Multiplex Ligation-dependent Probe Amplification)*. It is a multiplex technique that allows a relative quantification of the number of copies of several dozen different regions. It is indicated when structural variants of one or a few genomic regions are suspected.

2. Cases of **mosaicism** represent an additional difficulty. Mosaicism can occur at the level of germ cells, somatic cells, or both. Genetic skeletal disorders that may be related to germinal mosaicism include osteogenesis imperfecta and Down syndrome[3]. The usual sequencing techniques (classical or NGS) are usually unable to detect mosaicisms in which less than 5-10% of the analyzed cell population present the mutation. Sometimes, in these cases, it is useful to repeat the studies that have been carried out with DNA extracted from blood cells in other samples, such as cells from the oral mucosa or from the skin.

3. Detection of epigenetic alterations (especially cytosine methylation) using sequencing techniques requires prior treatment of DNA with bisulfite, which converts cytosines to uracils, while methylated cytosines remain unchanged. To detect the methylation status of specific genomic sequences, a variant of MLPA known as MS-MLPA (Methylation-Specific MLPA) is also used, which combines the use of MLPA with that of restriction enzymes that allow detecting whether the DNA sequence is methylated or not.

4. The aforementioned techniques (except for the sequencing of the whole genome) do not usually allow the identification of mutations that affect **regulatory regions** that are outside the coding region, nor some of those that cause alterations in the splicing (process of cutting and elimination of intronic regions in RNA and exon regions joining to form mature messenger RNA). If the latter are suspected, not genomic DNA should be sequenced, but rather that synthesized in vitro from RNA (cDNA).

5. Most sequencing and genotyping procedures are aimed at examining genomic DNA. In case of suspicion of an alteration of the **mitochondrial DNA**, procedures specifically directed to this end are needed[4].

6. Genetic tests are usually carried out on blood samples, which are easily accessible and useful for detecting germ-line mutations, that is, those present in all tissues of the body. However, as mentioned, some processes are due to **somatic or postzygotic mutations**, so that only some cells carry the mutation. In these cases, it is necessary to carry out the study in the affected tissue, since the results will be normal in the blood and other non-involved tissues.

Osteoporosis and other processes with decreased bone mass

In the vast majority of patients with osteoporosis, the disease appears in older adults or the elderly. It is the result of the interaction between genetic predisposing factors and environmental factors, together with skeletal deterioration induced by the decrease in sex hormones and other phenomena associated with it. aging. In general, susceptibility has a polygenic basis, determined by several tens or hundreds of genetic variants that, although with limited functional influence in isolation, together have a notable influence on bone mass. Thus, genome-wide association studies (GWAS) and some candidate gene association studies have identified more than 500 loci associated with bone mineral density or risk of fracture[5-7]. Efforts are being made to try to combine these loci into indices (often referred to as polygenic risk indices) that help determine individual risk of osteoporosis[8]. However, its applicability to the clinic is still very limited.

Occasionally, osteoporosis is the result of a specific mutation that has a marked functional impact and alters a gene with an essential role in skeletal homeostasis. Among the "monogenic" forms of osteoporosis, cases due to mutations in the LRP5, WNT1, DKK1 or PLS3 genes have been described[5].

Cases of juvenile or childhood osteoporosis, as well as those with a particularly strong family history, especially if they appear at an early age, are more likely to be due to point mutations. Genetic studies in juvenile and young adult osteoporosis have not consistently established a genetic basis. However, in some patients mutations have been detected in genes involved in skeletal homeostasis, in particular, some related to the Wnt pathway, such as LRP5, WNT1 or DKK1, or in collagen synthesis[9]. Therefore, in these patients it may be interesting to analyze a panel of genes that includes those most frequently related to skeletal disorders. Of course, before the genetic study it is indicated to rule out that osteoporosis is secondary to other systemic disorders (malabsorption, hyperthyroidism, etc.).

If there is no secondary cause of osteoporosis, it is also worth making sure that there is no other genetic disorder associated with osteoporosis. Among them, the most important is osteogenesis imperfecta. It presents with fragility fractures and, in some cases, bone deformities and blue or gray scleras. In most cases it is due to mutations in the genes that encode the alpha and beta

chains of collagen type 1 (COL1A1 and COL1A2), so the study can begin by analyzing these genes. In a recent series of 364 patients with various clinical forms of OI, 50-66% had COL1A1 mutations and 18-37% had COL1A2 mutations. However, in 20% of the cases, no mutations were found in the type 1[10] collagen genes. These cases may be due to mutations in non-analyzed regulatory regions, but mutations in other genes, which are also associated with lesser forms, must be ruled out. frequent symptoms of osteogenesis imperfecta[11,12] (Table 2).

In any case, one must always pay attention to the presence of other associated manifestations that suggest conditions in which osteoporosis is part of a systemic or syndromic process (for example, Turner syndrome, neurofibromatosis type 1, Marfan syndrome, etc.), which require a different diagnostic approach.

Osteopetrosis and other disorders with osteosclerosis

Disorders associated with osteosclerosis are much less common than those associated with decreased bone mass. Along with some acquired (osteoblastic metastases, myelofibrosis, fluorosis, etc.), others have a genetic origin[13]. They can be generalized or localized.

Among the forms of generalized osteosclerosis, osteopetrosis is the most common disorder. It may be due to mutations in several of the genes with an important role in osteoclastic activity, especially CLCN7 and TCIRG1 (which encodes the proton transporter ATPase). Other disorders that present with diffuse increase in bone mass are sclerosteosis and Van Buchem's disease, due to mutations in the SOST gene, which encodes sclerostin[14]. Probably the most effective approach to the genetic study of diffuse osteosclerosis includes the analysis of a panel of genes that includes those most frequently involved in these processes (Table 3). If the results are negative, the next step would be the sequencing of the entire exome.

The forms of localized osteosclerosis include various disorders, the diagnosis of which is generally based on the clinical and radiographic characteristics of the process, but whose genetic basis is generally not well established[15].

Mineral metabolism disorders

The approach to identifying the genetic basis for inherited disorders of mineral metabolism depends on the specific disorder in question.

Hypocalcemia

The most common genetic cause is pseudohypoparathyroidism, due to a loss of function of the GNAS gene[16,17]. This gene, which encodes a protein related to the G protein signaling pathway, is characterized by having a genetic imprint. That is, in most tissues the alleles transmitted by the two parents are expressed, but in some (such as the kidney, the pituitary, the gonads or the thyroid) only the maternal allele is expressed. Therefore, the inactivating mutations of the maternal allele cause resistance to PTH and other hormones, as well as a characteristic skeletal phenotype (short stature, rounded face, short metacarpals), which constitutes the so-called "hereditary Albright osteodystrophy". However, when the mutated allele is of paternal origin, osteodystrophy appears, but without hormonal alterations (pseudo-pseudohypoparathyroidism). The alterations of the GNAS gene that cause these conditions are of various types, including point mutations, structural abnormalities and alterations in the usual methylation patterns. Therefore, in suspicious cases, if the conventional analysis of the GNAS sequence does not reveal anomalies, the study should be deepened by resorting to other techniques (MLPA and MS-MLPA, above all)[18].

Table 2. Genes involved in monogenic forms of osteoporosis and osteogenesis imperfecta

Genes related to collagen synthesisand maturation	COL1A1 COL1A2 CRTAP PPIB P3H1 FKBP10 PLOD2 SERPINH1 BMP1
Genes related to other matrix proteins or osteoblastic activity	SPARC SERPINF1 IFITM5 PLS3 TMEM38B WNT1 SP7 (osterix) CREB3L1 MBTPS2 TENT5A (FAM46A) CCDC134

Table 3. Genes causing some processes that occur with increased bone density gen disease inheritance pattern

Gen	Disease	Pattern of inheritance
CLCN7	Osteopetrosis	AD/AR
TCIRG1	Osteopetrosis	AD/AR
CA2	Osteopetrosis	AR
OSTM1	Osteopetrosis	AR
SNX10	Osteopetrosis	AR
LRP4, LRP5	Various forms of hyperostosis	AD/AR
SOST	Sclerosteosis, Van Buchem disease	AD/AR
CTSK	Pycnodysostosis	AR
FAM20C	Raine syndrome	AR
GJA1	Oculodentodigital dysplasia	AD/AR
LEMD3	Osteopoikilosis	AD
TGFB1	Camurati-Engelmann disease	AD

Hypercalcemia

Genetic causes of hypercalcemia include familial hypocalciuric hypercalcemia, which is usually due to inactivating mutations in the gene that encodes the calcium

sensing channel (CASR). In case of suspicion, then, the study will begin with the analysis of this gene. If CASR mutations are not detected, the study will be extended to other genes (GNA11, AP2S1), which are responsible for a third of the cases. Interestingly, activating mutations of the CASR or GNA11 genes give rise to rare hypocalcemia of autosomal dominant inheritance[19].

Familial hyperparathyroidism may occur within a syndrome of multiple endocrine neoplasms (MEN1 or 2, often with abnormalities in the MEN1 or RET genes, respectively) or in isolation. The genetic basis of these latter cases is not always known, but some patients have mutations in the HRPT2 gene (CDC73) or in the CASR.

Hypophosphatasemia
The persistent decrease in alkaline phosphatase levels, in the absence of anti-resorptive treatment or other acquired causes that explain it, should lead to suspect an alteration of the ALPL gene, which encodes non-tissue-specific alkaline phosphatase (bone, liver). Therefore, in these cases the initial study should be aimed at sequencing said gene. Mutations in this gene give rise to hypophosphatasia, which can have serious phenotypic repercussions when it occurs in children, but is usually much milder if it occurs in adults. In fact, many cases in adults are asymptomatic or have only mild, nonspecific symptoms. However, detailed study may reveal subtle remodeling alterations that may be associated with an increased risk of adverse effects with anti-resorptive drugs[20,21].

Hypophosphatemia and other rickets
Family tree analysis usually gives very important information about the type of inheritance. The most common form of inherited rickets is X-linked hypophosphatemic rickets, due to mutations in the PHEX gene. Autosomal inherited rickets include, among others, those due to mutations in genes related to vitamin D (vitamin D-dependent rickets), such as those encoding renal hydroxylase involved in the synthesis of 1,25-dihydroxyvitamin D (CYP27B1) and the vitamin D receptor (VDR). Other autosomal inherited rickets are due to mutations in the FGF23, DMP1, ENPP1, and SCLC34A3[22] genes.

Developmental disorders

Many developmental disorders include abnormalities in the growth or shape of the bones. In some cases, they are part of complex syndromes, involving multiple organs and systems. The detailed study of the phenotype is essential to focus the genetic study. In many cases, these disorders are due to alterations in large chromosomal regions that affect several genes, in which case the first step may be aimed at identifying structural alterations, using karyotype, CGH arrays, or SNP arrays. Chondrodysplasias are a large group that includes more than 350 disorders in which the alteration of endochondral or endomembranous ossification causes often serious alterations of the skeleton[23,24]. They may or may not be accompanied by injuries at other levels. The phenotype can be very indicative in some cases with typical characteristics (for example, in achondroplasia) and the diagnosis can be confirmed by the targeted analysis of one or a few genes. However, in other cases with a less characteristic phenotype, it will be necessary to study, using massive sequencing procedures, a large panel of about 50-100 genes, or even to sequence the entire exome.

Localized lesions

Some single or multiple focal skeletal lesions may also have a genetic cause.

Paget's disease
Although Paget's disease can show a familial aggregation, in most cases the gene responsible for genetic susceptibility is not identified. However, in some cases it may be the result of point mutations in genes related to the sequestosome (SQSTM1/p62) or the RANKL pathway. Sequestosome mutations are identified in approximately 25-40% of familial Paget cases and 4-8% of sporadic cases[25,26]. On the other hand, there are forms of juvenile Paget's disease and other conditions with skeletal and extraskeletal involvement due to mutations in the TNFRSF11A and TNFRSF11B genes (encoding RANK and osteoprotegerin, respectively)[27].

Multiple exostoses
Patients with multiple exostoses develop excretory lesions (osteochondromas) at the level of the metaphyses from the first years of life. They can be asymptomatic, cause pain or affect growth, especially of the long bones of the extremities. It is generally due to mutations in the EXT1 gene, or, less often, the EXT2 gene, with autosomal dominant inheritance[28].

Multiple enchondromatosis
It also occurs with multiple skeletal lesions in children and young people, but, unlike exostoses (osteochondromas), enchondromas typically grow inside the bone. The cause is not firmly established, but somatic mutations of the PTHR1, IDH1, or IDH2[29] genes have been found in the enchondromas of some patients.

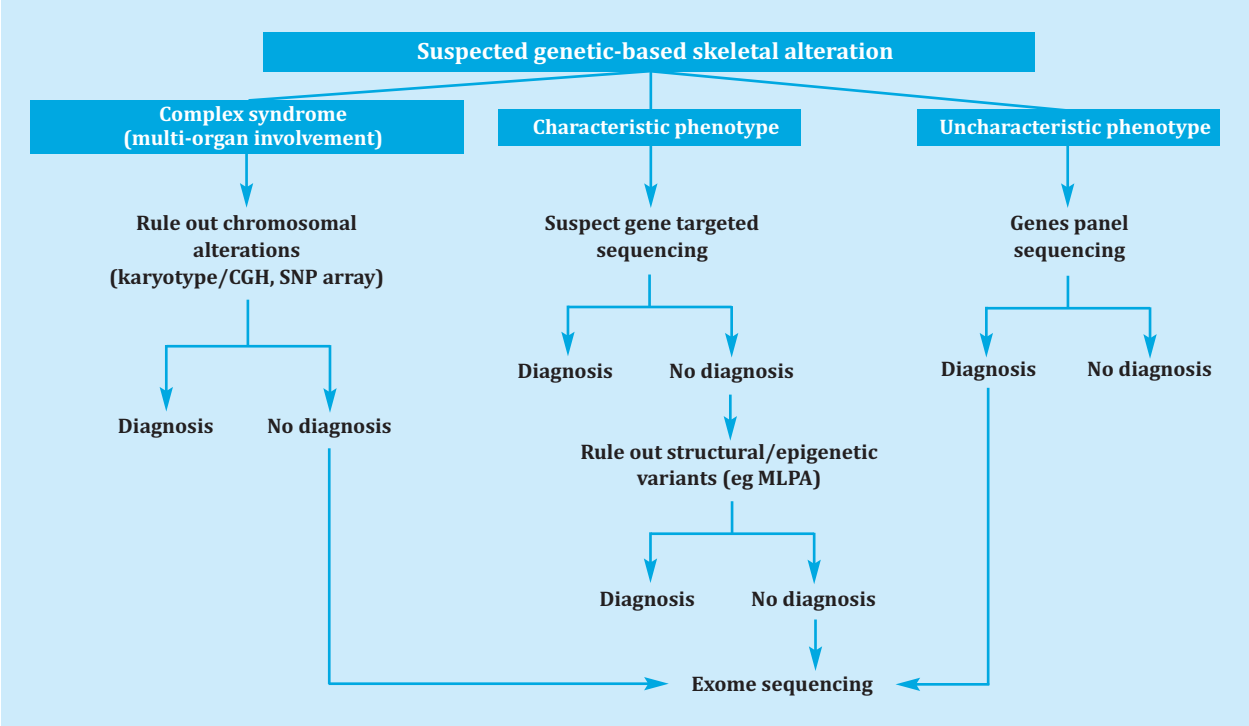
Fibrous bone dysplasia
Fibrous bone dysplasia can manifest as single or multiple bone lesions. Sometimes it is part of McCune-Albright syndrome, which also includes hyperpigmented skin macules and/or alterations due to an increase in the production of some hormones (precocious puberty, hyperthyroidism, hypercortisolism, excess GH). Excessive production of FGF23 in lesions makes hypophosphatemia a frequent manifestation. It is due to an activating somatic (postzygotic) mutation in the GNAS gene, which encodes a protein involved in the G protein signaling pathway[30]. Therefore, if a genetic study is to be carried out, it must be done with affected tissue, since the result in blood cells is usually normal.

Conclusion

The study of the genetic cause of a disorder depends on the suspected condition based on a detailed analysis of the phenotype. One of the first questions to answer is whether a disorder is suspected due to a mutation affecting a gene, or a broader chromosomal alteration. In the first case, the approach usually begins with the sequencing of the suspicious gene or genes, while in the second case, the procedures that allow the detection of structural variants, in general some type of array, are indicated.

If a specific genetic alteration is suspected, sequencing can focus on a gene, on a panel of related genes, or on the entire exome, depending on whether a gene is clearly suspicious, that is, a condition with genetic heterogeneity (Figure 1). The specific strategies depend not only on the condition under study, but also on the

Figure 1. Initial approach to skeletal disorders of genetic cause



availability of the diagnostic tests and protocols implemented in each center. However, it is likely that in the coming years, when costs are lowered and analytical procedures streamline, whole-exome sequencing will progressively replace gene panels. In fact, already at this moment, the realization of "virtual panels" can be more efficient in many cases[31]. In other words, the entire exome is sequenced, although at first only the existing variants in the genes potentially related to the phenotype are analyzed, thus reducing the number of mutations to be assessed by bioinformatics and literature analysis. However, since the entire exome was actually sequenced, if the initial analysis of the selected genes does not reveal pathogenic mutations, the study can be extended to other genes, without the need to sequence the sample again.

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