I- Introduction

The recognition of some of the most common genetic disorders is essential for clinical practice, not only to establish a definitive diagnosis, but also to propose a specific treatment and genetic counseling in order to reduce the risk of recurrence. An early diagnosis improves the management of the patient, resulting in less clinical problems, reducing morbidity, mortality, and its social impact.

Genetics has experienced great progress after the rediscovery of Mendel’s law, and the knowledge of the DNA base pair complementarity structure. New and powerful biological tools have been developed to identify the etiology and the diagnosis of many genetic diseases, and their use resulted in the finding of other pathological mechanisms of inheritance besides the classical ones (chromosomal aberrations, monogenic or mendelian diseases, and complex etiology of multifactorial diseases). As a result, other non-traditional forms, have been recognized, such as: microdeletions, microduplications, mosaicism, uniparental disomy, “imprinting” and mitochondrial inheritance.

Thus, to perform the diagnosis of chromosomal abnormalities, new powerful tests, such as MLPA (Multiplex ligation-dependent probe amplification) and CMA (Chromosomal Microarray), played an important role in the detection of microdeletions and microduplications. On the other hand, the study of genes, formerly done one by one using the Sanger sequencing technique, may now be done using a different technique (NGS – Next Generation Sequencing). It is even possible to study the complete exome (WES - Whole Exome Sequencing) and genome. Despite it presenting some limitations, many new genes and several diseases have been discovered by this method.\(^1\)

As a result, pathological mechanisms of some syndromes that were not very obvious before or even unidentified, have been elucidated. New genes remain to be discovered and a large number of diseases may now be identified by using specific tests, resulting in an extremely more appropriate and special way of managing these patients.

In view of this scenario, otolaryngologists should become more familiar with the “genetic language” that currently exists in medical fields. It is also important to recognize, as early as possible, the main genetic diseases involving the ear/hearing loss. In this sense, supplementary information can be found on “Hereditary hearing loss and its Syndromes” by Toriello, Reardon, Gorlin (2004)\(^2\); Atik \textit{et al} (2015)\(^3\); Koffler, Ushakov, Avraham (2015)\(^4\); and Parker & Gildicz (2016)\(^5\). The active participation of the ENT specialist in agreement with the geneticist and/or a multidisciplinary team has a positive influence in both the management as well as the course of the disease, leading to undoubted benefits not only to the individual, but also to the entire community.
Congenital anomalies

In 1980, infant mortality due to congenital anomalies in Brazil hovered around 5% and ranked in the fifth position. Nowadays, it occupies the second position; and in the city of São Paulo, congenital anomalies are considered the first cause of infant mortality.\(^6\)

Approximately, 3-5% of pregnancies result in the birth of a child with some type of congenital anomaly or genetic disease that will compromise their development and quality of life. In Brazil, the cases of pediatric hospitalization due to congenital malformations are estimated at 37%.\(^7\) For this reason, congenital anomalies and genetically determined diseases are considered one of the major public health problems.

The pathophysiological understanding of congenital anomalies is important to establish not only a definitive diagnosis, but also to better draw-up its management and genetic counseling.\(^8\)

Congenital anomalies can be isolated or associated with other defects, constituting multiple anomalies. Those isolated are subdivided into malformations, deformations, disruptions and dysplasias, while the associated ones are known as multiple syndromes, sequences and associations.\(^9\)

The primary morphogenetic errors are responsible for the malformations, whose severity depends on which organs and/or systems are affected. An extrinsic factor that compromises structures embryologically related and well developed are named deformities. On the other hand, disruptions are caused by injuries like vascular insufficiency, trauma or teratogens. The term Syndrome is reserved for situations where multiple malformations are pathogenetically related or the genetics etiology is defined.\(^9\)

A sequence is a group of congenital anomalies caused by events triggered by a primary event. It can be isolated or associated, such as the Pierre-Robin sequence, which constitutes an example in which a mandibular hypoplasia hinders the migration of the lateral palatine processes. Added to glossoptosis and the interposition of the tongue, this results in the formation of a cleft palate.\(^10\)

Association is defined by the concomitant occurrence of various malformations, more frequently than expected by chance. However, this diagnosis should be a diagnosis of exclusion. A classic example is the Vater association (V = vertebral anomalies, A = anal atresia, T = tracheoesophageal fistula, E = esophageal atresia, and R = renal and/or radial defects), whose acronym were expanded to VACTERL, because of the presence of cardiac (C) and limb anomalies (L).\(^11\) These mechanisms are not always clear, so that the CHARGE Syndrome, as it is currently known, and whose acronym covers the following anomalies: coloboma, heart defects, choanae atresia, retardation of growth and/or development, genital abnormality, ear anomalies and/or deafness, was formerly regarded as an association.

Genetic diseases are divided classically into three main broad groups, according to their etiology.

Complex etiology of diseases (multifactorial) resulting from the interaction of multiple genes with environmental factors;

Chromosomal: caused by chromosomal aberrations;
Monogenic: arising predominantly from changes in one gene, subdivided into autosomal dominant, autosomal recessive and X-linked.

**Diseases of complex etiology or multifactorial diseases**

Complex diseases are a group of diseases resulting from the interaction of environmental with genetic factors, contributing to the development of a certain phenotype.

This group includes most of the isolated malformations (e.g.: congenital heart disease, neural tube defects), and the most common adult diseases, such as: hypertension, atherosclerosis, diabetes, and obesity.

Some isolated ear anomalies / hearing deficits have a multifactorial etiology. In this chapter, we will focus on the congenital anomalies and syndromes with chromosomal and monogenic etiologies.

**Chromosomal Aberrations**

The human being has 46 chromosomes, 22 pairs of non-sex chromosomes, known as autosomes and one pair of sex chromosomes (XX in women and XY in men). The genes are DNA sequences packed into chromosomes located in the nuclei of cells. The location of the gene on the chromosome is known as locus. Alleles are alternative forms of a gene at that locus.

Chromosomal abnormalities account for a significant proportion of individuals with congenital anomalies and/or intellectual disability. They are divided into numerical and structural chromosomal anomalies. The numerical is characterized by an increasing or decreasing number of chromosomes in the cells. In humans, these abnormalities are subdivided into: a) polyploidy, when there is one or more additional complete sets of 23 chromosomes as, for example, triploidy – 69 chromosomes, or tetraploidy – 92 chromosomes; b) aneuploidy, when the total number of chromosomes is not a multiple of a set of 23 chromosomes, with loss (monosomy) or gain (trisomy, tetrasomy) of one or more chromosomes.

Structural chromosomal abnormalities take place when a break in the DNA molecule occurs, and rearrangements take place after the intervention or repair mechanisms are used, and may result in loss of genetic material (unbalanced chromosomal changes), or not (balanced). This group of structural anomalies is constituted by, for example, deletions, duplications, inversions, and translocations.

Chromosomal abnormalities are a group of genetic diseases in which various organs and systems are involved. For this reason, when we are faced with any congenital anomaly it is imperative to investigate and to search for others.

In patients with chromosomal abnormalities, besides hearing loss, frequently the ears are low-set and show a wide range of dysmorphisms. The most common chromosomal aberrations involving the ear/hearing loss are described in Table 1.

The diagnosis of chromosomal abnormalities is performed by a chromosomal study with banding, named Karyotype (Figure 1A). It can detect changes involving at least 5 Mb and is used routinely, especially in the Brazil’s National Health System (SUS – *Sistema Único de Saúde*). To perform a blood karyotype, 3 to 5 mL of peripheral blood in a tube containing heparin is required (green cap tube). This is an extremely simple procedure, but in some situations of severity and early death, unfortunately, in practice, it ends up being neglected because of the numerous emergency actions that have to be taken.
Table 1. Chromosomal Aberrations involving the ear/hearing loss

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<tr>
<th>Syndrome</th>
<th>Findings</th>
<th>Ears</th>
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<tbody>
<tr>
<td><strong>Down Syndrome</strong></td>
<td>Trisomy 21. Neuromotor delay, brachicephaly, up slanting palpebral fissures, epicanthal folds, short nose with low nasal bridge, protruding tongue, redundant nuchal skin, congenital heart defects, hip dysplasia, single transverse palmar crease, clinodactyly, wide gap between the first and second toes. Incidence: 1:800 live births, life expectancy ~ 60 years. Mortality in infancy depends on the presence of cardiac defects, leukaemia and respiratory disease.</td>
<td>Low set and small ears, angulated and folded-over upper helix, hearing loss</td>
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<td><strong>Edwards Syndrome</strong></td>
<td>Trisomy 18. Low birth weight, sucking difficulties, hypotonia followed by hypertonia, marked growth deficiency, prominent occiput, short sternum, congenital heart defects, kidney and others organ abnormalities, distinctive hand posture with overriding fingers, rocker bottom feet with shortened and flexed big toes. Frequency: 1 per 8,000 live births. The prognosis is quite reserved because by the presence of several associated malformations. About 50% die within the first month of life. Only 5% to 10% survive beyond the first year of life and, in general, have severe intellectual disabilities.</td>
<td>Dysmorphic and low-set ears.</td>
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<td><strong>Patau Syndrome</strong></td>
<td>Trisomy 13. Triad: microphthalmia, cleft lip/palate, polydactyly (70%), low birth weight, growth deficiency, aplasia cutis in parietal-occipital region, microcephaly, cleft lip/palate and involvement of many others organs/systems anomalies, such as: central nervous system, cardiovascular, digestive and urogenital. Frequency: 12 per 1,000 live births poor prognosis and survival is similar to that one of Trisomy 18.</td>
<td>Dysmorphic and low-set ears</td>
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<tr>
<td><strong>Wolf-Hirschhorn Syndrome</strong></td>
<td>4p deletion. Pre and post-natal growth deficiency, microcephaly, severe intellectual disability, hypotonia, seizures, typical craniofacial features as ‘Greek warrior helmet’ (wide bridge of the nose continuing to the forehead), prominent glabella, widely spaced eyes, strabismus, epicanthus, short philtrum, cleft lip/palate, involvement of many others organs/systems anomalies. Frequency 1 per 50,000 live births.</td>
<td>Poorly formed ears with pits/tags. Hearing loss (40%), mostly conductive</td>
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Cri-du-Chat Syndrome | 5p deletion. Sharp and weak cry like a cat meowing in the first days of life, growth retardation and development, microcephaly, ocular hypertelorism, epicanthus, broad nasal bridge, cleft lip and palate, micrognathia, cardiac defects, gastrointestinal and skeletal abnormalities. Frequency: 1:50,000 live births. | Poorly rotated ears with pits/tags.

Turner Syndrome | Monosomy X. Short stature, low-set hair neck, short and webbed neck, broad chest with spaced nipples, and hypoplastic, cubitus vlagus, transiente limphedema of hands and feet, gonadal dysgenesis, structural renal anomalies (horseshoe kidney) and heart defects (coarctation of the aorta). | Prominent and low-set ears

Some genetic Syndromes are associated with very small deletions or duplications that are undetectable by traditional chromosomal studies with bands, so called microdeletions or microduplications syndromes.

The phenotype of these microdeletions syndromes is caused by the deletion of contiguous genes leading to their haploinsufficiency.

Among the microdeletions Syndromes, 22q deletion and Williams Syndrome are the most frequent in the clinical genetics practice. Because of their supravalvular aortic stenosis Williams Syndrome patients usually are referred by a cardiologist. The otolaryngologists will find that these patients exhibit a peculiar cognitive profile. They are overfriendly and quite talkative, have a mild to moderate intellectual disability and visuospatial difficulties, which contrast with their special and unusual musical abilities. Some have absolute pitch, strong hyperacusis, and hearing loss.9

The main aberrations and chromosomal microdeletions involving the ear/hearing loss can be seen in Tables 1 and 2, respectively.

The diagnosis of microdeletions requires the use of techniques of molecular cytogenetic, such as FISH, MLPA or CMA.

Fluorescence in situ hybridization is a cytogenetic technique that can detect deletions of targeted fragments with less than 5 Mb (Figure 1B). This technique is performed in metaphase chromosomes, using a probe labeled with radioactive or fluorescent dyes that will hybridize and thus identify its supplementary segment. It is generally used for a specific region. Thus, to carry out this technique it is necessary to have a clinical suspicion that leads to testing a specific region.
Table 2. Microdeletions Syndromes most frequently associated with ear abnormalities

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<th>Syndrome</th>
<th>Clinical findings</th>
<th>Ear</th>
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<tr>
<td>22q11.2 Deletion Velocardiofacial and DiGeorge Syndrome</td>
<td>Facial dysmorphisms, such as telecanthus, narrow palpebral fissures, short philtrum, cleft lip/palate, velopharyngeal dysfunction, micrognathia, timic hypoplasia/aplasia, immune deficiency, parathyroid hypoplasia/aplasia (hypocalcemia and precocious seizures), particularly conotruncal malformations, including interrupted aortic arch and truncus arteriosus, slender extremities and elongated fingers with hyperextensible joints. 22q11.2 deletion is relatively common with an estimated incidence of approximately 1:2,000-4,000 live births. The clinical spectrum is extremely variable, and only psychiatric disorders may be present (schizophrenia and depression) without facial dysmorphisms.</td>
<td>Dysplastic ears</td>
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<tr>
<td>7q11.23 Deletion Williams Syndrome</td>
<td>Growth deficiency, typical facies (periorbital fullness, stellate pattern of iris, depressed nasal bridge, anteverted nares, long philtrum, prominent lips with open mouth), cardiovascular abnormalities (supravalvular aortic stenosis), neuromotor delay, overfriendliness, intellectual disability, which can range from mild to severe, recurrent otitis, hyperacusis, kidney, urinary and skeletal abnormalities, transient hypercalcemia, peculiar cognitive profile with a visuospatial difficulties that contrast with some cognitive abilities (musical ability). Some have absolute pitch. Deafness in some cases.</td>
<td>Hyperacusis in some cases is a prominent feature (the patients cover their ears or try to get away from the noise). Some have absolute pitch. Deafness in some cases.</td>
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</table>

Figure 1. A) G-banding karyotype of a patient with Edwards Syndrome (Trisomy 18). B) FISH showing the 7q11.23 microdeletion, compatible with Williams Syndrome.

On the other hand, MLPA allows the detection of microdeletions and microduplications of several chromosomal regions simultaneously using specific commercial kits.
CMA offers many advantages: it does not require neither a culture of dividing cells nor a prior karyotyping. It takes a comprehensive analysis of all chromosomes capable of detecting genomic microarrays from different known platforms.

In most developed countries the initial investigation is carried out primarily by an array, and the karyotype and FISH investigations are more commonly used to confirm the findings. In our country (Brazil), however, the high costs of CMA limits its use as a first-tier test.

**Monogenic diseases**

Monogenic diseases arise from a gene defect that can be autosomal or X or Y-linked. The location where the mutation is observed and whether it is present in one or both copies of the gene defines the mode of inheritance of these diseases, which can be: a) autosomal – when mutations occur in an autosomal chromosome, subdivided in dominant (in one copy of the gene) or recessive (in both copies of the gene); and b) X or Y-linked (holandric) when located on X or Y chromosome, respectively.

Today, genetic diseases are cataloged in a database known as Online Mendelian inheritance in man (OMIM), whose electronic version is available on the internet.\(^5\)

Ear anomalies, such as appendices, cysts or pits, require a complete genetic clinical investigation, in order to search for a syndromic etiology, especially if there are other malformations in other organs/systems, as well as dysmorphisms, a positive family history, and deafness.

Dysmorphisms are not always quite obvious or referred in the first medical evaluation, especially the auricular ones, making it difficult at times to recognize a genetic disorder. The absence of findings in the preliminary assessment, and the fact that the deafness is sometimes progressive, constitute other factors that make it difficult to diagnose this syndrome.

Hearing loss can be classified in different ways according to the: type (sensorineural, conductive and mixed); severity (mild, moderate, severe or profound); early age (prelingual and post-lingual); audiometric profile (downward-sloping, or low and high frequency, etc.), etiology (environmental or genetic, including syndromic and non-syndromic); and side (unilateral or bilateral). Hearing loss associated with the involvement of at least another organ/system defines “syndromic deafness”\(^5\).

Recent studies estimate that 1% of all human genes plays a role in hearing, and mutations in more than 80 genes have been reported to be responsible for non-syndromic deafness, a quarter of which have been discovered in the last five years.\(^3,5\) It is estimated that 30% of cases of sensorineural hearing loss are of syndromic etiology.\(^5\)

It is essential to identify the genomic alterations responsible for the disease, in order to establish the diagnosis and to perform an adequate management and genetic counseling to prevent a possible recurrence risk for future offspring of the couple. Many syndromes frequently observed in pediatric practice are associated with ear anomalies/hearing loss, such as: Stickler, Treacher Collins, Branchio-oto-renal, CHARGE, and Waardenburg Syndromes (**Table 3**).
<table>
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<tr>
<th>Syndrome</th>
<th>Clinical findings</th>
<th>Ear</th>
<th>Gene(s)</th>
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<tr>
<td>Treacher-Collins</td>
<td>Symmetric and bilateral abnormalities of the ears with meatus atresia, cleft lip/palate, lower eyelid coloboma and sparse, partially absent, or totally absent lashes, hypoplasia of the mandible and the zygomatic complex, preauricular hair displacement onto the cheeks, unilateral or bilateral choanal stenosis or atresia. Intelligence is normal. Incidence: 1: 50,000.</td>
<td>Symmetric and bilateral abnormalities of the ear which can be absent, small, and rotated, atresia or stenosis of the external auditory canals (36%) and conductive hearing loss (40%-50%) attributed to ankylosis, hypoplasia, or absence of the ossicles and hypoplasia of the middle ear cavities.</td>
<td>TCOFI (71% - 93%), POLR1 or POLR1D (8%)</td>
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<td>OAV (Oculoauriculovertebral spectrum, Goldenhar Syndrome)</td>
<td>Developmental disorder involving structures derived from the first and second pharyngeal arches during embryogenesis. Heterogeneous phenotype, of variable severity, with ear abnormalities (preauricular pits or tags, dysplastic ears, anotia, microtia, with or without deafness), hemifacial microsomia, with facial asymmetry, ocular abnormalities (epibulbar dermoids, microphthalmia, upper eyelid coloboma), and vertebral anomalies. Incidence: 1:3,500 live births.</td>
<td>Preauricular pits or tags, dysplastic ears, anotia, microtia, with or without deafness</td>
<td>Unknown</td>
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<tr>
<td>Branchio Oto Renal</td>
<td>Major criteria: second branchial arch anomalies, deafness, preauricular pits, auricular malformations, and renal anomalies (67%). Minor criteria: external auditory canal anomalies, middle ear anomalies, inner ear anomalies, preauricular tags, facial asymmetry, and palate abnormalities. Incidence: 1:40,000.</td>
<td>Deafness: mild to profound in severity; conductive, sensorineural, or mixed, preauricular pits or tags, auricular malformation, middle ear abnormalities: malformation, malposition, dislocation, or fixation of the ossicles; reduction in size or malformation of the middle ear space, cochlear hypoplasia; enlargement of the cochlear and vestibular aqueducts; hypoplasia of the lateral semicircular canal, external auditory canal atresia or stenosis.</td>
<td>EYA1 (40%), SIX5 (5%), SIX1 (4%)</td>
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<td>Beckwith-Wiedemann</td>
<td>Accelerated growth, macrosomia, macroglossia, visceromegaly, embryonal tumors such as Wilms tumor, hepatoblastoma, neuroblastoma, and rhabdomyosarcoma, omphalocele, neonatal hypoglycemia, renal abnormalities. Estimated prevalence of 1: 13,700</td>
<td>Ear creases/pits</td>
<td>Cytogenetic; epigenetic and genomic alterations of chromosome region 11p15; and CDKN1C mutation (5%-10% - familial cases)</td>
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<td>CHARGE</td>
<td>Coloboma (80% -90%), heart defects (75% -85%), choanal atresia (50-60%), growth deficiency (70% -80%) and developmental delay, genital hypoplasia (50% -60 %), ear abnormalities (especially aplasia of the semi-circular canals), cranial nerve abnormalities. Incidence: 1: 12,000</td>
<td>Small and dysmorphic ears (&quot;cup-shaped&quot;), deafness (sensorineural, mixed or conductive). Mondini defect, hypoplastic or absent semicircular canals</td>
<td>CHD7 (&gt;90% in typical and 65%-75% of atypical cases), SEMA3E</td>
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<td>Townes-Brocks</td>
<td>Triad: imperforate anus (84%), dysplastic ears, frequently associated to deafness and abnormal thumb (89%), which can be triphalangeal, duplicated or more rarely hypoplastic, renal impairment (42%), heart defects (25%), foot malformations (52%) and genital abnormalities (36%). Intellectual disability occurs in approximately 10% of individuals.</td>
<td>Dysplastic ear (87%) and deafness; &quot;Satyr ear&quot; with overfolded superior helices and preauricular tags or pits; frequently associated with sensorineural and/or conductive hearing impairment (65%)</td>
<td>SALL1</td>
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<tr>
<td>Otopalatodigital</td>
<td>Otopalatodigital spectrum disorders include the Otopalatodigital syndrome type I (OPD1), Otopalatodigital syndrome type II (OPD2), Frontometaphyseal dysplasia (FMD), Melnick-Needles syndrome (MNS), and Terminal osseous dysplasia with pigmentary skin defects (TOD). All of them have X-linked inheritance. The mildest manifestations occur in males with OPD1: cleft lip/palate, mild skeletal abnormalities, and conductive deafness attributed to ossicles abnormalities. FMD shows a generalized skeletal dysplasia, deafness and urogenital defects. OPD2 males present skeletal dysplasia and several anomalies in hindbrain, heart, intestines and kidneys, which frequently lead to perinatal death. MNS is the most severe phenotype with a skeletal dysplasia in the heterozygote. Affected males exhibit severe malformations similar to those observed in individuals with OPD2, resulting in prenatal lethality or death in the first few months of life.</td>
<td>Mixed hearing loss in all Syndromes of the spectrum, except TOD</td>
<td>FLNA</td>
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<td>Stickler</td>
<td>Connective tissue disorder with inter and intrafamilial variable expressivity, which seems to be secondary to locus and allele heterogeneity. Patients present myopia, cataract, retinal detachment, midfacial underdevelopment, cleft/lip palate (isolated or part of Pierre-Robin sequence), mild spondyloepiphyseal dysplasia with or without precocious arthritis.</td>
<td>High frequency conductive and sensorineural hearing loss</td>
<td>COL2A1, COL1A1, COL1A2, COL9A1, COL9A2, COL9A3</td>
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<td>Distrofic displasia</td>
<td>Limb shortening, adducts thumbs (&quot;hitch-hiker&quot;), spinal deformities (scoliosis, exaggerated lumbar lordosis and cervical kyphosis). It can be lethal at birth, but most affected survive the neonatal period, presenting significant physical limitations with normal intelligence.</td>
<td>Cystic ear swelling in the neonatal period (in ~2/3 of infants with the classic findings)</td>
<td>SLC26A2 (&gt;90%)</td>
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<td>Waardenburg</td>
<td>Auditory-pigmentary syndrome characterized by pigmentary abnormalities of the hair, skin, and eyes; congenital sensorineural hearing loss; and lateral displacement of the ocular inner canthi. Most patients have a white forelock before 30 years of age, most common in Type I. Affected patients may exhibit segmental or total iris heterochromia. Iris can be brilliant blue and hypoplastic. Congenital leukoderma is frequently observed on the trunk or extremities. <strong>Type I</strong>: Dystopia canthorum, iris heterochromia, white forelock, sinophis, nose with alar hypoplasia <strong>Type II</strong>: Without dystopia canthorum <strong>Type III</strong>: Klein-Waardenburg syndrome: <strong>Type I +</strong> upper limbs anomalies <strong>Type IV</strong>: Waardenburg-Shah syndrome: Waardenburg + Hirschsprung disease</td>
<td>The hearing loss in WS1, observed in approximately 60% of affected individuals, is congenital, typically non-progressive, either unilateral or bilateral, and sensorineural. Most commonly, hearing loss in WS1 is bilateral and profound (&gt;100 dB).</td>
<td>Type I: PAX3 (90%) Type II: PAX3, SNAI2, WS2B, WS2C, SOX10, MITF Type III: PAX3 Type IV: EDNRB, EDN3, SOX10</td>
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In order to establish the definitive diagnosis of a monogenic disorder, it is necessary to identify the causative gene(s) mutation(s). The Sanger sequencing technique has been used since the 1980s. Its major applicability is related to diseases with responsible genes that have few exons. However, when the gene has a large number of exons, the Sanger technique is a time-consuming and laborious procedure.
A powerful diagnostic tool emerged after the Human Genome Project: Next Generation Sequencing – NGS. Using this technique it is possible to perform a conjoint analysis of several genes simultaneously from a single blood sample. Thus, it allows the study of a group of genes related to a clinical finding or disease (gene panel)\(^{19}\) or the study of the coding regions (exons) of all the genes described in the human genome, known as Whole-Exome Sequencing (WES). \(^{20},^{21}\) The gene panel, even though with lower cost than WES, has the limitation of studying only the genes already described as related to the suspected disease.\(^{19}\) On the other hand, WES includes all the genes, raising the possibility of identifying new genes related to a disease. Although this method has revolutionized the field of Medical Genetics, it still has some limitations, such as insufficient coverage of coding exons. Moreover, the interpretation of the pathogenicity of a large number of variants found in each individual (between 20,000-30,000) is a difficult task, despite the use of numerous filters to try to identify the mutations that are responsible for the phenotype. Recent studies indicate that WES has a diagnostic yield of 20-30%.\(^{20,21}\)

A variant can be classified as: a) pathogenic; b) possibly pathogenic; c) benign (polymorphisms); d) possibly benign; and e) uncertain meaning variants (Variants of uncertainty clinical significance – VUS or VOUS).\(^{22}\) Due to the uncertainty of the pathogenicity in some situations, as well as the high cost of the exam, it should be noted that the molecular study may be inconclusive, making it difficult to arrive to a definitive diagnosis.

**References**