GENETIC COUNSELLING IN VISUAL AND AUDITORY DISORDERS

EL ASESORAMIENTO GENÉTICO EN LOS DÉFICITS VISUALES Y AUDITIVOS

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RESUMEN

Objetivo: Las enfermedades hereditarias que afectan a la retina y la audición presentan una amplia heterogeneidad clínica y genética. Durante la pasada década se han producido importantes avances en el conocimiento de la patogenia molecular de estas enfermedades y, actualmente, más de 200 genes y loci están implicados en enfermedades de la retina y más de 60 son responsables de pérdida de audición.

Método: El estudio genético molecular es crucial para confirmar el diagnóstico clínico, permite, en ocasiones, conocer el pronóstico de la enfermedad, un consejo genético y reproductivo adecuado y permite la posibilidad de crear grupos de pacientes genéticamente homogéneos para futuros ensayos clínicos.

Resultados: El elevado número de genes implicados hace que el diagnóstico molecular no sea factible en términos de coste, tiempo y esfuerzo técnico, y no existe ningún centro que oferte el análisis de todos los genes conocidos. Recientemente, se han desarrollado varias herramientas diagnósticas dirigidas a paliar este problema.

ABSTRACT

Purpose: Inherited retinal dystrophies and hearing loss disorders have a broad clinical and genetic heterogeneity. Over the last decade there have been major advances in our understanding of the molecular pathology of these diseases; currently over 200 genes and loci are known to be involved in retinal disorders, and over 60 genes/loci are causative for hearing impairment.

Methods: Genetic testing is crucial for confirming the diagnosis at a molecular level. It also allows a more precise prognosis to be made of the future clinical evolution, as well as an accurate genetic and reproductive counselling, and raises the possibility of creating genetically homogeneous groups of patients for future clinical trials.

Results: The high number of genes responsible for these disorders makes molecular testing overwhelming in terms of cost, time and technical effectiveness, and no centre offers testing of all known genes. Several diagnostic tools have emerged recently to circumvent this problem.

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**INTRODUCTION**

Diseases derived from genetically induced loss of vision and hearing constitute one of the most important problems for public health systems. In addition, these diseases affect organs which are necessary for communication and therefore involve a high degree of social isolation.

An increasing amount of couples visit the ophthalmology, ENT or genetics practices searching for counseling due to family histories of retinopathy or hearing problems which are beginning to affect one of the members of the couple or one of their children who has had one of these disorders.

**SUBJECTS, MATERIAL AND METHODS**

**Genetic counseling**

Genetic counseling consists in providing available medical and scientific information to patients affected by a hereditary disease or who are at risk of suffering or transmitting said disease to their descendants. Genetic counseling also includes measures for treating or delaying the symptoms of the disease and preventing its transmission. The geneticist does not counsel the patient; rather, his or her task is to provide information for the patient to make a decision. Genetic counseling involves:

1. Obtaining a diagnostic of the disease. This is the first step, which is sometimes difficult to achieve, particularly in neurosensory diseases because occurrences such as invariable expressiveness (to be explained below) are a factor in this type of disease. Without an adequate clinical diagnostic, genetic counseling will be incomplete and lacking in precision.
2. Risk estimations, both for developing the disease and transmitting it to descendants.
3. Specialized assistance, including consultations with specialist physicians, psychological assistance in some occasions and the offer of a prenatal or pre-implant genetic diagnosis.

The third point involves molecular genetic analysis. Prenatal genetic diagnostic involves the detection of the mutations which are responsible for the disease in the embryo by means of a chorial biopsy (between weeks 11-13 of gestation) or amniocentesis (week 15-16).

The pre-implant genetic diagnosis involves implanting in the uterus embryos which are free of the disease to avoid therapeutic abortion. It consists in the in vitro fecundation of ova (previously extracted from the mother-to-be) with sperm of her partner. When the embryo has between six and eight cells, 1 of them is extracted and analyzed for the genetic defect which was previously detected in the couple. The only embryos to be implanted will be those that do not have the defect.

For this prenatal or pre-implant diagnostic to be possible, it is first necessary to know which is the genetic defect involved in the family: not only which gene is altered but also the specific mutations of this gene which are present in the patient.

As will be described below, genetic tests are not simple and, in the case of neurosensory diseases, on many occasions cannot be carried out with the means available at this time.

Initially, it was believed that a disease (a clinical entity) was caused by a single gene. Accordingly, searches were initiated for genes such as the gene of pigmentary retinosis, the Usher syndrome gene, the
maculopathy gene, etc. Subsequently, the development of molecular genetics has evidenced in the past two decades an enormous genetic heterogeneity for both hypoacusis and blindness, which means that genetic counseling in families having a history of these diseases, particularly in sporadic cases, is very difficult.

The progress made in the research of the genes and pathogenic mechanisms involved in these diseases do not match the possibilities for studying them in public health labs. In fact, to date over 180 genes involved in syndromic and non-syndromic retinal degenerations have been identified. Retinal dystrophies constitute the core of hereditary problems related to eyesight due to their prominence and complexity and the absence of treatment. Accordingly we shall now focus on these dystrophies. By way of example, a clinical entity such as pigmentary retinosis (PR) exhibits the three Mendel patterns of hereditary transmission. To date, 14 genes have been identified for the dominant autosomal forms, 19 for the recessive autosomal form and 2 linked to chromosome X; for Leber’s congenital amaurosis, 15 genes have been found and at least one more is assumed to be still unidentified. In addition, a transmission pattern can be autosomal dominant or recessive. Nine different genes have been identified for the Bardet-Biedl syndrome (1), a further 9 for Usher syndrome (2), a disease associating both visual and auditory deficit (discussed in this paper) and at least 2 for Refsum disease, a rare peroxisomal disease which also involves deafness with PR in addition to peripheral neuropathy, anosmia, ataxia cerebelloso and in some cases skeletal displasia, icotiosis, cataracts and heart arrhythmia (3). The cause of this disease is the accumulation of phytanic acid due to failure in its degradation process.

In the context of this overview of retinal dystrophies, we must note the broader diversity of functions carried out by the proteins involved (see table I). Of all these genes, a certain number encode proteins involved in phototransduction, including rhodopsine, alpha and beta subunits of phosphodiesterase dependent on cyclic GMP, the alpha and beta sub-units of the ionic channel dependent on cyclic GMP specific for rods, arresteine, etc.

Others intervene in the cycle of vitamin A and are involved in the process of transport and isomerization of 11-cis-retinal to all-trans-retinal, either inside the photoreceptor or in the retinal pigmentary epithelium (RPE). We have comprised these proteins within the metabolism of the visual cycle, including the MERTK protein, involved in phagocytosis of opsine discs of the external segments of fault receptors in the RPE because, even if it is not directly part of the protein machinery which accounts for the metabolism of retinoids, it does form part (just like the other proteins) of the processes which take place between the external segment of the photoreceptor and the RPE.

A third function represented by genes involved in retinopathies is the development of the retina in itself; for example, CRX and NRL transcription factors which seemed to be involved either individually or synergistically in regulating the expression of retina-specific genes such as rhodopsine.

Recently, it was proved that mutations in some genes involved in the pre-RNA messenger processing produce RP. It is interesting that these genes, PRPF3, PRPF8 and PRPF31, expressed ubiquitously throughout the body while causing a pathological phenotype only in the retina.

The largest percentage of genes involved in retinal dystrophies have a structure or function. These proteins are involved in the correct arrangements of three-dimensional structures of the photoreceptor, the opsine discs (RDS-peripherin and ROM1), the connecting cilium and the peri-ciliar region (TULP1, USH2A, CRB1, FSCN2, RP2, RPGR, RPGRIP1) important for the traffic of proteins from the internal to the external segments, anchoring of the photoreceptor to the RPE, etc.

In addition, some genes are involved in the integration of visual information, control of apoptosis and other as yet unknown functions.

As regards neurosensory hypoacusis, over 25 genes have been identified as being involved in the non-syndromic forms and over 20 in syndromic forms, rendering it virtually impossible in approximately half of all cases to trace the gene or genes involved in the disease of a specific family.

Table II shows the main genes involved in a hereditary non-syndromic hypoacusis, their chromosomic localization, their function and whether these genes are also responsible for some other form of syndromic hypoacusis as occurs with retinopathies.

In addition, there are still many of the genes involved in these pathologies which are already
Table I. Listing of genes identified as responsible of some retinopathies, showing only the genes known to date as being involved in pigmentary retinosis, Leber’s congenital amaurosis, macular dystrophies (excluding age-related macular dystrophies) and cone and rod dystrophies, their chromosomal localization, the proteins they encode and the function they carry out. Column 5 shows the percentage of cases due to each gene in which a molecular epidemiological study has been carried out. The percentages shown do not include the prevalence of each gene within all retinal degenerations but only of the clinical subtype which were screened for mutations. Thus, RPGR accounts for 75% of cases of pigmentary retinosis linked to chromosome X, rhodopsine accounts for 19-25% of autosomal dominant pigmentary retinosis, etc.

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Protein</th>
<th>Function</th>
<th>%</th>
<th>Disease (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNGA1</td>
<td>4p12</td>
<td>Ionic channel dependent on cGMP alpha sub-unit</td>
<td>Photo-transduction</td>
<td>2,2</td>
<td>ARRP (30)</td>
</tr>
<tr>
<td>CNGB1</td>
<td>16q13</td>
<td>Ionic channel dependent on cGMP beta sub-unit</td>
<td>Photo-transduction</td>
<td></td>
<td>ARRP (31)</td>
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<tr>
<td>GUCA1B</td>
<td>6p21.1</td>
<td>Guanine activator cyclase 1B</td>
<td>Photo-transduction</td>
<td>20</td>
<td>ADRP, domMD (32)</td>
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<tr>
<td>RHO</td>
<td>3q22.1</td>
<td>Rhodopsine</td>
<td>Photo-transduction</td>
<td>19-25</td>
<td>ADRP, ARRP, DCSNB (33-34)</td>
</tr>
<tr>
<td>PDE6A</td>
<td>5q33.1</td>
<td>alpha sub-unit of phosphodiesterase of cyclic GMP of rods</td>
<td>Photo-transduction</td>
<td>4</td>
<td>ARRP (35)</td>
</tr>
<tr>
<td>PDE6B</td>
<td>4q16.3</td>
<td>beta sub-unit of phosphodiesterase of cyclic GMP of rods</td>
<td>Photo-transduction</td>
<td>4</td>
<td>ARRP, DCSNB (36)</td>
</tr>
<tr>
<td>SAG</td>
<td>2q37.1</td>
<td>Arrestin</td>
<td>Photo-transduction</td>
<td>2.9</td>
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<td>CNGB3</td>
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<td>Subunit 3 of cationic channel specific to cones</td>
<td>Photo-transduction</td>
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<td>KCVN2</td>
<td>9p24.2</td>
<td>Member 2 of subfamily of sodium channel Proteins</td>
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<td>RCOD (39)</td>
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<td>RDS15</td>
<td>12q13.2</td>
<td>11-cis-retinol dehydrogenase 5</td>
<td>Visual cycle metabolism</td>
<td>2.9</td>
<td>DCORD (40)</td>
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<tr>
<td>ABCA4</td>
<td>1p22.1</td>
<td>Member of family of ATP binding cassette</td>
<td>Visual cycle metabolism</td>
<td>2.5</td>
<td>ADRP, RMD, RCORD (41)</td>
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<td>IMPDH1</td>
<td>7q32.1</td>
<td>Inosine monophosphate dehydrogenase 1</td>
<td>Visual cycle metabolism; metabolism of guanine</td>
<td>0.6</td>
<td>ADRP, RLCA (42)</td>
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<tr>
<td>MERKT</td>
<td>2q13</td>
<td>Thyrosine kinase proto-oncogene C-mer</td>
<td>Visual cycle metabolism</td>
<td></td>
<td>ADRP (43)</td>
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<tr>
<td>RGR</td>
<td>10q23.1</td>
<td>Receptor associated to retina-specific Protein G</td>
<td>Visual cycle metabolism</td>
<td>0.5</td>
<td>ADRP, choroidal sclerosis (44)</td>
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<tr>
<td>RLBP1</td>
<td>15q26.1</td>
<td>Protein 1 for joining to retinaldehyde</td>
<td>Visual cycle metabolism-RPE</td>
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<td>ADRP (45)</td>
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<tr>
<td>BEST1</td>
<td>11q12.3</td>
<td>Bestrofie-1</td>
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<td>domMD (Best type) (46)</td>
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<td>RPE65</td>
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<td>RPE Protein 65</td>
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<tr>
<td>CA4</td>
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<td>Carbon anhydrase 4</td>
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<td>4</td>
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<tr>
<td>RDH12</td>
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<tr>
<td>IDH3B</td>
<td>20p13</td>
<td>Beta sub-unit of dehydrogenase isocitrate specific for mitochondrial NAD(+)</td>
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<tr>
<td>ELOVL4</td>
<td>6q14.1</td>
<td>Elongase-4 of long chain fatty acids</td>
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<td>DCORD (52)</td>
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<tr>
<td>PITPNM3</td>
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<td>Member 3 of the Protein family which transport phosphatidyl-inositol through the membrane</td>
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<tr>
<td>CRX</td>
<td>19q13.32</td>
<td>Homeobox of cones and rods</td>
<td>Retinal development</td>
<td>1</td>
<td>ADRP, DLCA, RLCA, DCORD (53)</td>
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<tr>
<td>NRL</td>
<td>14q11.2</td>
<td>Leucine, retina chain</td>
<td>Retinal development</td>
<td>0.7</td>
<td>ADRP, ARRP (54)</td>
</tr>
<tr>
<td>LRA7</td>
<td>4q32.1</td>
<td>Lecithin-retinol acyltransferase</td>
<td>Retinal development</td>
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<tr>
<td>NR2E3</td>
<td>15q23</td>
<td>Nuclear Receptor, subfamily 2, group E, member 3</td>
<td>Retinal development</td>
<td></td>
<td>ADRP (56)</td>
</tr>
</tbody>
</table>
Table I. Listing of genes identified as responsible of some retinopathies, showing only the genes known to date as being involved in pigmentary retinosis, Leber’s congenital amaurosis, macular dystrophies (excluding age-related macular dystrophies) and cone and rod dystrophies, their chromosomal localization, the proteins they encode and the function they carry out. Column 5 shows the percentage of cases due to each gene in which a molecular epidemiological study has been carried out. The percentages shown do not include the prevalence of each gene within all retinal degenerations but only of the clinical subtype which were screened for mutations. Thus, RPGR accounts for 75% of cases of pigmentary retinosis linked to chromosome X, rhodopsine accounts for 19-25% of autosomal dominant pigmentary retinosis, etc. (continuation)

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<th>Function</th>
<th>%</th>
<th>Disease (reference)</th>
</tr>
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<tbody>
<tr>
<td>CEP290</td>
<td>12q21.32</td>
<td>Centrosomic Protein of 290KD</td>
<td>Retinal development; connecting cilium</td>
<td>21</td>
<td>ARRP, RLCA, JS (57)</td>
</tr>
<tr>
<td>EYS</td>
<td>6q12</td>
<td>EYS orthologic Drosophile</td>
<td>Retinal development</td>
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<td>ARRP (58)</td>
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<tr>
<td>PROM1</td>
<td>4p15.32</td>
<td>Prominine</td>
<td>Retinal development. Morphogenesis of opsin discs</td>
<td></td>
<td>DCORD, domMD (59)</td>
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<td>PRPF3</td>
<td>1q21.3</td>
<td>Pre-mRNA processing factor 3</td>
<td>mRNA processing</td>
<td>1</td>
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<td>PRPF8</td>
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<td>PRPF31</td>
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<td>mRNA processing</td>
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<td>TOPORS</td>
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<td>Union factor for topoisomerase rich in argynin/serin</td>
<td>IMRNA processing, ubiquitination</td>
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</tr>
<tr>
<td>RD3</td>
<td>1q32.3</td>
<td>RD3 Protein</td>
<td>MRNA processing</td>
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</tr>
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<td>RAX2</td>
<td>19p13.3</td>
<td>Homeobox 2 transcription factor</td>
<td>MRNA processing</td>
<td></td>
<td>CORD (64)</td>
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<td>FSC2</td>
<td>17q25.3</td>
<td>Fascine 2</td>
<td>Structural: Maintenance of cytoskeleton of photoreceptors</td>
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<td>ADRP (65)</td>
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<td>RDS</td>
<td>6p21.2</td>
<td>RDS/peripherin</td>
<td>Structural: Maintenance of disc structure</td>
<td>9.5</td>
<td>ADRP, domMD, RP digenic with ROM1 (42)</td>
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<td>ROM1</td>
<td>11q12.3</td>
<td>Protein of membrane of external segment of photoreceptors</td>
<td>Structural: Maintenance of disks (digenic with RDS)</td>
<td>2</td>
<td>RP digenic with RDS (42)</td>
</tr>
<tr>
<td>RP1</td>
<td>8q12.1</td>
<td>RP1</td>
<td>Structural: Communication/ traffic of external-internal segment through CC</td>
<td>3.5</td>
<td>ADRP, ARRP (66)</td>
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<tr>
<td>TULP1</td>
<td>6p21.31</td>
<td>Protein 1 tubby-like</td>
<td>Structural: Communication/ traffic of external-internal segment through CC</td>
<td>2</td>
<td>ADRP, RLCA (67)</td>
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<tr>
<td>USH2A</td>
<td>1q41</td>
<td>Usherin</td>
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<td>ADRP, USH (68)</td>
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<tr>
<td>CRB1</td>
<td>1q31.3</td>
<td>Crumb homologue</td>
<td>Structural: Anchoring to extra-celular matrix</td>
<td>6.5</td>
<td>ADRP, RLCA (69)</td>
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<tr>
<td>RP2</td>
<td>Xp11.23</td>
<td>RP2</td>
<td>Structural: Communication/ traffic of external-internal segment through CC</td>
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<td>XLRP (70-71)</td>
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<tr>
<td>RPGR</td>
<td>Xp14</td>
<td>GTPase Regulator</td>
<td>Structural: Communication/ traffic of external-internal segment through CC</td>
<td>75</td>
<td>XLRP, XLCORD, XLCNSB (70-71) RLCA (72)</td>
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<tr>
<td>RPGRIP1</td>
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<td>RP Protein associated to GTPase receptor</td>
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<td>LCA5</td>
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<td>Lebercilin</td>
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<tr>
<td>SEMA4A</td>
<td>1q22</td>
<td>Semaphrin 4ª</td>
<td>Neurosensory integration</td>
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<tr>
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<td>RLCA, DCORD (72)</td>
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<tr>
<td>RIMS1</td>
<td>6p13</td>
<td>Rim-1</td>
<td>Neurosensory integration</td>
<td></td>
<td>DCORD (75)</td>
</tr>
<tr>
<td>CACNA2D4</td>
<td>12p13.33</td>
<td>Alpha sub-unit-2/delta-4 of calcium channel</td>
<td>Neurosensory integration</td>
<td></td>
<td>RCOD (76)</td>
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</tbody>
</table>
identified, i.e., the chromosome region where they are located is known but they still have not been characterized and therefore their mutational screening is not possible.

To follow the ongoing process of updating of identified and localized genes for the various forms of hereditary transmission of hypoacusis, we suggest visiting the Hereditary Hearing Loss Home-page: http://webh01.ua.ae.be/hhh/
and RetNet for retinal diseases: http://www.sph.uth.tmc.edu/Retnet/

### RESULTS

Considering current possibilities and tools and the exceptions to Mendel’s laws of inheritance proven by experience, we detail below a brief summary of the possibilities of studying the aforementioned diseases.

### Genetic tests which can be performed by health system labs

The studies offered in health system labs depend on a number of factors such as:

The number and complexity of the genes involved in the disease. As discussed above, in the case of retinal dystrophies and hypoacusis, both factors have a very negative effect on the possibility of performing simple genetic tests.

Perhaps due to the anatomical and physiological complexity of both the sight and hearing organs and to the amount of proteins and genes required for their correct performance, the detection of the genetic defect which is responsible for poor performance in each patient is a very arduous task.

Within the complexity of both problems there is an important difference between hypoacusis and retinopathies. In the former, 80% of hereditary cases correspond to a recessive autosomal pattern. In addition, 5 mutations, 35delG in gene GJB2, of (D13S1854) and of (D13S1830) in gene GJB6, mutation 1555G>A of the mitochondrial r12S gene and mutation Q829X of the OTOF gene are present in about 50% of hypoacusis cases in Spain (4) and therefore a relatively simple screening of these five mutations allows genetic counseling for a considerable number of families with hypoacusis history.

For retinopathies the case is a lot more complicated. There is no transmission pattern as prevalent as with hypoacusis or any gene which accounts for a high percentage of retinopathies (5).

These problems make the study of these diseases dependent on funding derived from research projects, not only as regards consumables but mainly staff.

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<tr>
<td>CERKL</td>
<td>2q31.3</td>
<td>Protein similar to TA ceramide Kinase</td>
<td>Control apoptosis</td>
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<td>AIPL1</td>
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<td>Protein associated to the aryl-hydrocarbon receptor</td>
<td>Chaperon</td>
<td>3.4 RLCA, DCORD (72)</td>
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<td>PRCD</td>
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</tr>
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ARRP: Recessive autosomic pigmentary retinosis; ADRP: Dominant autosomic pigmentary retinosis; XLRP: pigmentary retinosis linked to Chromosome-X; CNB: stable congenital nycalopia (dominant DCSNB, XLCNBN: linked to chromosome-X); LCA: Leber’s congenital Amaurosis (DLCA: dominant, RLCA: recessive); MD: macular dystrophy (domMD: dominant, RMD: recessive); CORD: cones and rods dystrophy (DCORD dominant, RCORD: recessive, XLCORD: linked to chromosome X); JS: Joubert syndrome; USH: Usher syndrome; CC: connecting cilium; cGMP: Cyclic Guanosine Monophosphate; RPE: retinal pigmentary epithelium.

Table I. listing of genes identified as responsible of some retinopathies, showing only the genes known to date as being involved in pigmentary retinosis, Leber’s congenital amaurosis, macular dystrophies (excluding age-related macular dystrophies) and cone and rod dystrophies, their chromosomal localization, the proteins they encode and the function they carry out. Column 5 shows the percentage of cases due to each gene in which a molecular epidemiological study has been carried out. The percentages shown do not include the prevalence of each gene within all retinal degenerations but only of the clinical subtype which were screened for mutations. Thus, RPGR accounts for 75% of cases of pigmentary retinosis linked to chromosome X, rhodopsine accounts for 19-25% of autosomal dominant pigmentary retinosis, etc. (continuation)
Problems derived from non-typical transmission forms: alteration of the theoretical recurrence risk

Both for retinal hereditary diseases as for neurosensory hypoacusis, there are examples of hereditary transmissions which follow the three classic Mendelian patterns; dominant autosomal forms, recessive autosomal forms and gender related forms. In addition, some forms of retinopathies and hypoacusis are associated to mitochondrial problems.

However, there is a number of events well known to geneticists which occur in this type of diseases and hereditary transmission models, adding to the complication of genetic counseling. These events include pleiotrophism, which occurs with relative frequency. Some examples of this event could be the majority of the genes involved in Usher’s syndrome which, depending on the type of mutation, involve hearing, the retina or both. As can be seen in table I, some genes can give rise to pigmentary retinosis, Stargardt’s disease and age related macular degeneration (6). Incomplete penetration is a frequent occurrence in Waardenburg syndrome (7). Variable expressiveness is also frequent in retinopathies as well as in hypoacusis. For instance, patients with the 35delG mutation in gene GJB2 exhibit hypoacusis which can range from slight to severe (8,9) or other groups of patients affected by pigmentary retinosis with mutations in the rhodopsine gene can exhibit a variable initial stage of the disease and a variable degree of visual field loss, even within members of
Another event which affects exclusively gender-linked forms is lyonization or random deactivation of chromosome-X. This could explain the fact that some carrier women exhibit symptoms of the disease which can be as severe as those of a male affected by a retinopathy linked to chromosome X. Less frequent are the cases of uni-parent dysomia but, even so, a case of partial uni-parent dysomia of chromosome 1 which gave rise to Stargardt disease in a patient (10,11) and a similar case in chromosome 13 gave rise to hypoacusis due to mutations in gene GJB2 in brothers of the same family (12). Finally, cases of di-genic inheritance have been detected in retinopathies between the peripherin/RDS and Rom-1 proteins, both part of the discs of external segments of retina photoreceptors (13) and hypoacusis between conexines 26 and 30 (14), although recently the di-genic inheritance model for these conexines is under question (15).

**DISCUSSION**

**Limitations and benefits of genetic counseling**

The main limitation is that derived of the complexity of genetic tests for detecting one or more mutations responsible for disease in each family. In addition there are problems derived from the possible variations of the Mendelian forms of transmission of the disease.

As discussed above, at present a health system genetics lab is unable to study a number of genes accounting for over 50% of hereditary hypoacusis and an even lower percentage of retinopathies. Even in specialized labs dedicated exclusively to these pathologies, the percentage of resolved cases is far from the totality. Until genetic tools are developed allowing for the study of a large number of genes and mutations in a short period of time and at a reasonable cost, this will not be possible.

**DNA Microchips**

One of said tools of recent appearance is the DNA microchip or micro-array, which facilitates the detection of a larger number of mutations in different genes simultaneously with a small sample of a patient’s DNA in a very short period of time (16-18). Utilizing conventional techniques, this work would take months. However, the drawback is that these microchips only detect the mutations which are incorporated to it and does not detect new mutations. Therefore, undescribed mutations in patients will go unnoticed by the microchip. To enhance the efficiency of the microchip it is necessary to carry out a mutational analysis of these patients by conventional methods and subsequently update the microchip to include the newly detected mutations.

Another tool of recent appearance is high-performance genotyping by means of SNPs (19). Through an indirect study, the SNPs microchip is able to discard a given number of genes involved in RP as well as in Leber’s congenital amaurosis. Depending on the size of the family, the number of members involved and the presence of consanguinity, the SNPs discards and the involvement of an important number of genes involved in these pathologies. This is very useful as a first diagnostic approach but is not useful in sporadic cases and does not avoid the sequencing of non-discarded genes.

A short-term benefit of the molecular diagnostic of retinal dystrophies it is the creation of a genetically homogeneous group of patients who can be subjected to clinical essays and specific gene therapy protocols in the future.

**Perspectives for therapy**

In the current absence of a therapy to prevent the development of the disease, the benefits of genetic studies resides not only in the possibility of prenatal diagnosis but also in the creation of genetically homogeneous groups of patients for clinical essays in the future.

It must be noted that research is focused on developing therapies from a variety of angles.

**Treatment of neurosensory hypoacusis**

The treatment of hearing loss is approached from different angles according to the degree and nature of the hypoacusis:

In patients exhibiting slight and moderate-severe hypoacusis, they refer improvement in hearing with the use of hearing aids.
In patients with congenital deep deafness and patience with progressive hypoacusis reaching severe-deep stages, cochlear implants are being applied with considerable success.

**Therapy perspective for retinal degenerations**

In contrast with hypoacusis, at present there is no pharmacological or prosthetic treatment for recovering the damaged organ although therapeutic strategies are being developed depending on the stage of the disease:

In the initial stages of the disease it is possible to apply therapies for slowing down the degeneration process via the application of neurotrophic factors, or curing strategies such as gene therapy or retinal transplants.

In the final stages in which the retinal degeneration is highly advanced, only retinal transplants or a visual implants will be useful.

**Neurotrophic factors**

These substances protect retinal cells from apoptotic processes which activate the death of retinal cells. It is necessary to administer these factors continuously and in physiological dosages. An outstanding development which is obtaining a great success in initial trials with humans is the utilization of the so-called *encapsulated cells*. A small capsule made of porous material containing neurotrophic factor-releasing cells is implanted in the ocular globe (20).

**Gene therapy**

This therapy consists in the substitution or repair of the defective genes by introducing genetic material in the cells by means of a vector.

The strategy should be different depending on the form of transmission of the retinopathy. For example, in dominant inheritance cases the expression of the mutant gene is to be suppressed, whereas in recessive inheritance cases a non-mutant gene should be included to achieve the correct expression thereof.

An additional difficulty for the application of gene therapy is their expression. Some genes involved in the disease have expression specifically in the retina. For these cases, the problem lies in the delivery of the gene to the retina going through the rest of ocular layers.

However, in many cases, the altered gene exhibits different isoforms and expresses in several different tissues, sometimes ubiquitously. On some occasions these genes are part of the machinery which accounts for the development and expression of other genes. Even though these genes are expressed in different tissues or systemically they only produce pathology in the retina, possibly because the alteration occurs only in the specific isoform of this tissue. However, other causes such as the presence of genes in tissues other than the retina (which substitute the function of the altered gene) cannot be discarded. For these cases, the difficulty lies in that the modification of the expression of this gene which is pathological in the retina could cause adverse effects in the function of the same gene in other tissues. It is necessary to determine the biological processes whereby a gene expresses a specific isoform in a tissue or at a given time of the development thereof in order to develop rational gene therapies.

A further problem is the low transduction efficiency of vectors. Finally, the under or over expression of some transgenes can give rise to a degeneration of photoreceptors (21).

Recent research indicates that the most efficient vectors for a stable integration of transgenes in the genome of retinal cells (and which also exhibits a high and persistent level of expression) are the *lentivirus* (22). These have been utilized in the first gene therapy experiments for retinal dystrophies carried out in the rd mutant mouse by means of the introduction of GFP, a fluorescent protein which can be easily identified in histological sections in retinal pigment epithelium sales (23). At present, 2 clinical essays are being carried out with gene therapy, 1 for age-related macular degeneration (24) and another one for Leber’s congenital amaurosis due to mutations in the *RPE65* gene (25). Both essays in stage one and have demonstrated the safety of the injection of certain viral vectors in the eye.

**Retinal transplants**

--- *Artificial retinae*: this is a minuscule microchip containing microscopic solar cells
which convert light into electrical impulses. The microchip is surgically implanted in a sub-retinal lateral of the patient’s eye. This technology is not yet applicable for curing visual deficiencies but research in this field is developing at a fast pace.

— Stem cells: these multi-potent cells can be cultured in labs and, with the right stimulation, are induced to differentiate into the desired cell population. In this case, the target is obtaining in vitro retinae for subsequent transplant (23). For a long time it was believed that the only adult tissues from which stem cells could be obtained were in the bone marrow, the epithelium of the skin, muscles and the digestive tract. Subsequently it was demonstrated that progenitor cells can be obtained from many other tissues, including the eye (26). This therapeutic strategy is supported by the fact that the retina is a privileged location from the immunological viewpoint. The delivery of cells and tissues to the retina has been proved to be clearly feasible and in some cases even beneficial (27). However, the attempts to compensate for the loss of photoreceptors by means of a subretinal transplant of fetal retinal layers has been disappointing (28). This type of transplant exhibits a low synaptic integration in the host retina and does not produce good results in patients with RP (29).

It has been demonstrated that the synaptic integration capacity largely depends on the state of differentiation of the transplanted cells. Recently, the group of Professor Robin Ali from London University demonstrated that post-mitotic photoreceptors integrated with greater efficiency in the mouse retina than the non-differentiated progenitor cells (23). This discovery evidences that, even though each one of these therapies is highly promising, it is still necessary to delve deeper in the biology of the retina to achieve rational therapies for retinal dystrophies.

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