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Genetic testing for cone rod dystrophies

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of the genetic test for cone rod dystrophies (CORDs). CORDs are caused by variations in the *ABCA4, ADAM9, AIPL1, C8orf37, CACNA1F, CACNA2D4, CDHR1, CNGA3, CRX, DRAM2, GUCA1A, GUCY2D, HRG4, KCNV2, PDE6C, PITPNM3, POC1B, PROM1, PRPH2, RAB28, RAX2, RIMS1, RPGRIP1, RPGR SEMA4A, TTLL5* genes, with an overall prevalence of 1 per 40 000. Most genes have autosomal recessive inheritance; the others have autosomal dominant or X-linked recessive transmission. Clinical diagnosis is based on clinical findings, color vision testing, ophthalmological examination and electroretinography. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

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Cone-rod dystrophies

(other synonyms: CORDs, CRDs, cone-rod degeneration, cone-rod retinal dystrophy, CRD, retinal cone-rod dystrophy)(Retrieved from ghr.nlm.nih.gov)

General information about the disease

Cone-rod dystrophies (CORDs) are a large and heterogeneous group of inherited disorders, affecting the photoreceptoral cells of the retina. They are characterized by low visual acuity (<1/20), nystagmus, photophobia, abnormal color vision, progressive night blindness and peripheral visual field loss (which occurs earlier than in retinitis pigmentosa) (1). CORDs are characterized by primary loss of cone photoreceptors followed by secondary loss of rod photoreceptors. They are typically classified as non syndromic and syndromic, the latter being one aspect of more complex conditions.

The prevalence of CORDs is estimated to be about 1 per 40 000 (2).

The diagnosis of CORDs is based on clinical findings, ophthalmological and instrumental examination, electroretinography and color vision testing. It is confirmed by identifying the pathogenic variants of the genes by molecular genetic testing.

Differential diagnosis should include other forms of inherited retinal dystrophie, such as retinitis pigmentosa and Leber congenital amaurosis and other diseases such as maculopathies and achromatopsia.

CORDs may be transmitted as an autosomal recessive trait associated with variations in the following genes: *ABCA4* (OMIM gene: 601691; OMIM disease: 604116), *ADAM9* (OMIM gene: 602713; OMIM disease: 612775), *AIPL1* (OMIM gene: 604392; OMIM disease: 604393), *C8orf37* (OMIM gene: 614477; OMIM disease: 614500), *CACNA2D4* (OMIM gene: 608171; OMIM disease: 610478), *CDHR1* (OMIM gene: 609502; OMIM disease: 613660), *CNGA3* (OMIM gene: 600053), *DRAM2* (OMIM gene: 613360; OMIM disease: 616502), *POC1B* (OMIM gene: 614784; OMIM disease: 615973), *PROM1* (OMIM gene: 604365; OMIM disease: 612657), *RAB28* (OMIM gene: 612994; OMIM

disease: 615374), *RPGRIP1* (OMIM gene: 605446; OMIM disease: 608194), *SEMA4A* (OMIM gene: 607292; OMIM disease: 610283), *TTLL5* (OMIM gene: 612268; OMIM disease: 615860), *KCNV2* (OMIM gene: 607604; OMIM disease: 610356), *PDE6C* (OMIM gene: 600827; OMIM disease: 613093), or as an autosomal dominant trait associated with variations in the following genes: *CRX* (OMIM gene: 602225; OMIM disease: 120970), *GUCA1A* (OMIM gene: 600364; OMIM disease: 602093), *GUCY2D* (OMIM gene: 600179; OMIM disease: 601777), *HRG4* (UNC119, OMIM gene: 604011), *PITPNM3* (OMIM gene: 608921; OMIM disease: 600977), *PROM1* (OMIM gene: 604365; OMIM disease: 612657), *PRPH2* (OMIM gene: 179605), *RAX2* (OMIM gene: 610362; OMIM disease: 610381), *RIMS1* (OMIM gene: 606629; OMIM disease: 603649) or as an X-linked trait associated with variations of *CACNA1F* (OMIM gene: 300110; OMIM disease: 300476) or *RPGR* (OMIM gene: 312610; OMIM disease: 304020) genes.

Pathogenic variants may include small intragenic insertions/deletions missense, nonsense, splice-site and deep intronic variations. Partial or whole gene deletions/duplications are also reported, mostly for the *ABCA4, AIPL1, CRX, KCNV2, PRPH2* and *RPGR* genes (3-5).

Aims of the test

- To determine the gene defect responsible for the pathology;
- To confirm clinical diagnosis of the disease;
- To determine carrier status for the disease.

Test characteristics

Experts centers/Published guidelines

The test is listed in the Orphanet database and is offered by more than 16 medical genetic laboratories in the EU, and in the GTR database, offered by about 16 medical genetic laboratories in the US.

The guidelines for clinical use of the test are described in "Genetics home reference" (ghr.nlm.nih.gov) and "Clinical Utility Gene Card" (6).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in *ABCA4, ADAM9, AIPL1, C8orf37, CACNA1F, CACNA2D4, CDHR1, CNGA3, CRX, DRAM2, GUCA1A, GUCY2D, HRG4, KCNV2, PDE6C, PITPNM3, POC1B, PROM1, PRPH2, RAB28, RAX2, RIMS1, RPGRIP1, RPGR SEMA4A,* and *TTLL5* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in *ABCA4, AIPL1, CRX, PRPH2* and *RPGR* genes. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have CORDs. To perform molecular diagnosis, a single sample of biological material is normally sufficient. This may be 1 ml blood in a sterile tube with 0.5 ml K3EDTA or 1 ml saliva in a sterile tube with 0.5 ml ethanol 95%. Sampling rarely has to be repeated. Gene-disease associations and interpretation of genetic variants are rapidly developing fields. It is therefore possible that the genes mentioned in this note may change as new scientific data is acquired. It is also possible that genetic variants today defined as of "unknown or uncertain significance" may acquire clinical importance.

Genetic tests results

Positive

Identification of pathogenic variants in *ABCA4, ADAM9, AIPL1, C8orf37, CACNA1F, CACNA2D4, CDHR1, CNGA3, CRX, DRAM2, GUCA1A, GUCY2D, HRG4, KCNV2, PDE6C, PITPNM3, POC1B, PROM1, PRPH2, RAB28, RAX2, RIMS1, RPGRIP1, RPGR SEMA4A* and *TTLL5* genes confirms the clinical diagnosis and is an indication for family studies.

A pathogenic variant is known to be causative for a given genetic disorder based on previous reports or predicted to be causative based on the loss of protein function or expected significant damage to protein or protein/protein interactions. In this way it is possible to obtain a molecular diagnosis in new/other subjects, establish the risk of recurrence in family members and plan preventive and/or therapeutic measures.

Inconclusive

Detection of a variant of unknown or uncertain significance: a new variation and/or without any evident pathogenic significance or with insufficient or significant conflicting evidence to indicate it is likely benign or likely pathogenic for a given genetic disorder. In these cases, it is advisable to extend testing to the patient's relatives in order to assess variant segregation and clarify its contribution. In some cases it could be necessary to perform further examinations/tests or to do a clinical reassessment of pathological signs.

Negative

The absence of variations in the genomic regions investigated does not exclude a clinical diagnosis but suggests the possibility of:

- alterations that cannot be identified by sequencing, such as large rearrangements that cause loss (deletion) or gain (duplication) of extended gene fragments;
- sequence variations in gene regions not investigated by this test, such as regulatory regions (5' and 3' UTR) and deep intronic regions;
- variations in other genes not investigated by the present test.

Unexpected

Unexpected results may come out from the test, for example information regarding consanguinity; absence of family correlation or the possibility of developing genetically based diseases.

Risk for progeny

In autosomal dominant transmission, the probability that a carrier transmits the disease variant to his/her children is 50%

in any pregnancy, independently of the sex of the conceived.

Autosomal recessive transmission needs that both healthy carrier parents transmit their disease variant to his/her children. In this case, the probability of having an affected boy or girl is therefore 25%.

In X-linked transmission, affected males only transmit the disease variant to their daughters. The probability that a female carrier transmits the pathogenic variant to her offspring is 50% in any pregnancy independently of the sex of the conceived. Females who inherit the pathogenic variant will be carriers and usually unaffected. Males who inherit the pathogenic variant will be affected.

Limits of the test

The test is limited by current scientific knowledge regarding the genes and disease.

Analytical sensitivity (proportion of positive tests when the genotype is truly present) and analytical specificity (proportion of negative tests when the genotype is not present)

NGS: Analytical sensitivity: >99% (with a minimum coverage of 10X); Analytical specificity: 99.99%.

SANGER: Analytical sensitivity: >99.99%; Analytical specificity: 99.99%.

MLPA: Analytical sensitivity: >99.99%; Analytical specificity: 99.99%.

Clinical sensitivity (proportion of positive tests if the disease is present) and clinical specificity (proportion of negative tests if the disease is not present)

Clinical sensitivity: variations in known causative genes are currently identified in 78% of cases. In most cases, the disorder is associated with variations in the *ABCA4* gene (24-65% depending on the population) (6).

Clinical specificity: is estimated at approximately 99.99% [Author's laboratory data] (7).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for the disease;

b) the genetic test has diagnostic sensitivity greater than or equal to other published tests.

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	yes
Differential diagnosis	yes
Access to clinical trial (8)	yes
Couple risk assessment	yes

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