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Genetic testing for ocular coloboma

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for ocular coloboma (COI). COI is inherited in an autosomal dominant manner associated with variations in the *PAX6*, *ABCB6* and *FZD5* genes and in an autosomal recessive manner associated with variations in the *SALL2* gene. Overall prevalence is 1 per 100,000 live births. Clinical diagnosis is based on clinical findings, ophthalmogical examination, family history, fundus 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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Ocular coloboma

(other synonyms: congenital ocular coloboma, uveoretinal coloboma)(retrieved from ghr.nlm.nih.gov)

General information about the disease

Ocular coloboma (COI) is a rare congenital eye developmental defect caused by failed optic fissure closure during embryo development. Various eye structures such as the iris, choroid, retina and optic nerve may be involved. Due to their embryological origin, colobomas are usually inferior, with manifestations varying from the simple lower iris fissure, to absence of a large portion of the lower portion of the retina. Involvement of the optic nerve is less frequent. The visual consequences vary according to the location and extent of the defect. Specifically, sight is generally conserved if the coloboma is limited to a small part of the iris, but may be impaired (with risk of retinal detachment) when the retina and optic nerve are involved. Some individuals with coloboma are affected by a condition called microphthalmia (1), in which one or both eyeballs are abnormally small. Other symptoms and signs that may be related to COI are cataract, heterochromia (2), glaucoma, myopia, nystagmus and retinal detachment.

The prevalence of COI is estimated at around 1 in 100 000 live births.

Diagnosis of COI is based on clinical findings, ophthalmogical examination, family history, fundus examination and electroretinography. It is confirmed by detection of pathogenic variants in causative genes.

Differential diagnosis should consider aniridia, heterochromia irides, iris nevi, iris trauma, iris atrophy, Rieger syndrome, morning glory disk, congenital optic pits and optic nerve staphylomata.

COI caused by variations in the *PAX6* (OMIM gene: 607108; OMIM disease: 120200), *ABCB6* (OMIM gene: 605452; OMIM disease: 614497) or *FZD5* (OMIM gene: 601723; Liu (3)) gene is inherited in an autosomal dominant manner. COI caused by variations in the *SALL2* (OMIM gene: 602219; OMIM disease: 216820)(4) gene is inherited in an autosomal recessive manner.

Pathogenic variants may contain small intragenic deletions/insertions as well as splice-site, missense and nonsense variants. Partial or whole gene deletions/duplications are generally not reported except to PAX6 (5).

Aims of 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 3 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 12 accredited medical genetic laboratories in the US.

The guidelines for clinical use of the test are described in "Genetics home reference" (ghr.nlm.nih.gov).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the *PAX6, ABCB6, FZD5* and *SALL2* genes. Potentially causative variants and regions low coverage are Sanger-sequenced. MLPA is used to detect deletions in PAX6 gene. Sanger sequencing is also used for family segregation studies. The tests identify variations in known causative genes in patients suspected to COI. 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 the 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 test results

Positive

Identification of pathogenic variants in *PAX6, ABCB6, FZD5* or *SALL2* 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:

- 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;
- alterations that cannot be identified by sequencing, such as large rearrangements that cause loss (deletion) or gain (duplication) of extended gene fragments.

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%.

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: Due to the limited number of cases described, there is currently no clinical sensitivity for ocular coloboma available in the literature.

Clinical specificity: data not available.

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 (7)	yes
Couple risk assessment	yes

References

1. Hornby SJ, Adolph S, Gilbert CE, Dandona L, Foster A. Visual acuity in children with coloboma: clinical features and a new phenotype classification system. Ophthalmology. 2000 Mar; 107: 511-20. PubMed PMID: 10711890.
2. Morrison DA, Fitz-Patrick DR, Fleck BW. Iris coloboma with iris heterochromia: a common association. Arch Ophthalmol. 2000 Nov; 118: 1590-91. PubMed PMID: 11074823.
3. Liu C, Widen SA, Williamson KA, Ratnapriya R, Gerth-Kahlert C, Rainger J, et al. A secreted WNT-ligand-binding domain of FZD5 generated by a frameshift mutation causes autosomal dominant coloboma. Hum Mol Genet. 2016 Apr; 25(7):1382-91. PubMed PMID: 26908622. Pubmed Central PMCID: PMC4787907. Epub 2016/01/24.
4. Kelberman D, Islam L, Lakowski J, Bacchelli C, Chanudet E, Lescai F, et al. Mutation of SALL2 causes recessive ocular coloboma in humans and mice. Hum Molec Genet. 2014 May 15; 23: 2511-26. PubMed PMID: 24412933. Pubmed Central PMCID: PMC3990155. Epub 2014/01/09.
5. Guo H, Dai L, Huang Y, Liao Q, Bai Y. A large novel deletion downstream of PAX6 gene in a Chinese family with ocular coloboma. PLoS One. 2013 Dec 11; 8(12):e83073. PubMed PMID: 24349436. Pubmed Central PMCID: PMC3859656.
6. Chen B, Gagnon M, Shahangian S, Anderson NL, Howerton DA, Boone JD. Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions. MMWR Recomm Rep. 2009 Jun 12; 58 (RR-6):1-37. PubMed PMID: 19521335.
7. Stone EM, Aldave AJ, Drack AV, Maccumber MW, Sheffield VC, Traboulsi E, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. Ophthalmology. 2012 Nov; 119(11):2408-10. PubMed PMID: 22944025. Epub 2012/09/01.