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Genetic testing for Mendelian glaucoma

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for Mendelian glaucomas, a large heterogeneous group of inherited disorders, classified according to age of onset as congenital glaucoma, juvenile glaucoma and age-related glaucoma. Variations in the *TEK, MYOC, ASB10, NTF4, OPA1, WDR36* and *OPTN* genes are inherited in an autosomal dominant manner and variations in the *CYP1B1* and *LTBP2* genes have autosomal recessive inheritance.

The prevalence of congenital glaucoma is estimated at 1-9 per 100 000, that of juvenile glaucoma at 1 per 50 000, while there is insufficient data to establish the prevalence of age-related glaucoma.

Clinical diagnosis is based on clinical findings, age of onset, family history, ophthalmological examination, intraocular pressure, gonioscopy and fundoscopy. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

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Mendelian glaucoma

(other synonyms: genetic glaucoma)

General information about the disease

Mendelian glaucoma (acronym MG) is a large and heterogeneous group of inherited disorders characterized by elevation of intraocular pressure (IOP), related to loss of retinal ganglion cells, and responsible for characteristic degeneration of the optic nerve and therefore severe impairment of the visual field.

Mendelian glaucoma is classified according to age of onset:

- congenital glaucoma present at birth
- juvenile glaucoma that develops in the first two decades of life
- age-related glaucoma that develops between 40 and 60 years of age.

or on a clinical basis:

- open-angle glaucoma (the most frequent)
- closed-angle glaucoma (caused by anatomical disorders and characterized by sudden ocular pain and nausea/vomiting)

or:

- high IOP
- normal IOP.

Mendelian glaucoma may be due to primary isolated defects or secondary to any of a large variety of metabolic and genetic syndromes, such as anterior segment dysgenesis syndromes (1, 2).

Clinically, all glaucomas are characterized by insidious progressive narrowing of the visual field, tearing. Some forms are associated to enlargement of the ocular globe (bu-

phthalmos), corneal edema and photophobia.

The prevalence of congenital glaucoma is estimated at 1-9 per 100 000; juvenile glaucoma has a prevalence of 1 per 50 000; there is insufficient data to establish the prevalence of Mendelian age-related glaucoma.

Diagnosis of MG is based on clinical findings, age of onset, family history, ophthalmological examination, visual field testing, IOP measurement, gonioscopy and fundoscopy. It is confirmed by identification of pathogenic variants in associated genes by molecular genetic testing.

Differential diagnoses should include segment dysgenesis syndromes, other forms of non-hereditary glaucoma, optic nerve disease, sequelae of obstetric trauma, metabolic disease and corneal diseases such as megalocornea.

While congenital and juvenile glaucoma affects relatively few individuals, age-related glaucoma is the leading cause of blindness throughout the world (3,4). Epidemiological data suggests that age-related forms are inherited as a complex trait, without any obvious segregation pattern, although recent studies have named specific causative genes (5,6). Juvenile and congenital forms are inherited as Mendelian traits with high penetrance.

MG has different patterns of inheritance. Variations in the *ASB10* (OMIM gene: 615054; OMIM disease: 603383), *MYOC* (OMIM gene: 601652; OMIM disease: 137750), *NTF4* (OMIM gene: 162662; OMIM disease: 613100), *OPA1* (OMIM gene: 605290; OMIM disease: 606657), *OPTN* (OMIM gene: 602432; OMIM disease: 137760),*TEK* (OMIM gene: 600221; OMIM disease: 617272), and *WDR36* (OMIM gene: 609669; OMIM disease: 609887) genes have autosomal dominant inheritance and variations in the *CYP1B1* (OMIM gene: 601771; OMIM disease: 231300) and *LTBP2* (OMIM gene: 602091; OMIM disease: 613086) genes have autosomal recessive inheritance.

The *CYP1B1, TEK, MYOC* and *LTBP2* (7-9) genes are associated with congenital glaucoma. Studies suggest that juvenile glaucoma is determined by variations in the *CYP1B1, MYOC, ASB10, NTF4, OPA1, WDR36* and *OPTN* genes.(10,11) Age-related glaucoma is mainly associated with variations in the *OPTN, WDR36, MYOC* and *CYP1B1* genes (5,6).

Pathogenic variants may include sequence variations: missense, nonsense, splicing, small insertions and deletions.

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

Expert centers/Published guidelines

The test is listed in the Orphanet database and is offered by 98 accredited medical genetic laboratories in the EU, and in the GTR database, offered by about 13 accredited medical genetic laboratories in the US.

The guidelines for clinical use of the test are described in "Genetics home reference".

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the *ASB10, CYP1B1, LTBP2, MYOC, NTF4, OPA1, OPTN, TEK,* and *WDR36* genes. Potentially causative variants and region with low coverage are Sanger-sequenced. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have MG. 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 the *ASB10, CYP1B1, LTBP2, MYOC, NTF4, OPA1, OPTN, TEK,* and *WDR36* 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%.

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%.

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 *CYP1B1* are reported in 20-100% of familial cases with primary congenital glaucoma and in 10-15% of simplex cases (12).

Variations in *MYOC*, *OPTN* and *WDR36* (taken together) are the genetic cause of less than 10% of cases of primary open angle glaucoma/juvenile open angle glaucoma.

10–20% of adult-onset *POAG* cases are due to pathogenic variants in the MYOC gene (9, 13).

Variations in *ASB10* are identified in about 5-7% of cases (14), while variations in the *NTF4* gene are reported in 1.7% of patients (15) Reazie et al. estimated that variations in *OPTN* are responsible for 16.7% of the hereditary forms of normal-IOP glaucoma (6).

Clinical specificity is estimated at about 99% [Author's laboratory data] (16).

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 (17)	yes
Couple risk assessment	yes

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