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Genetic testing for Mendelian cataract

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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 Mendelian cataract (MC). MC is caused by variations in the *AGK, BFSP1, BFSP2, CHMP4B, CRYAA, CRYAB, CRYBA1, CRYBA2, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD, CRYGS, EPHA2, EYA1, FYCO1, FOXE3, FTL, GALK1, GCNT2, GJA3, GJA8, HSF4, LEMD2, LIM2, LSS, MAF, MIP, NHS, PITX3, PAX6, SIPA1L3, SLC16A12, TDRD7, UNC45B, VIM, VSX,* and *WFS1* genes. The overall prevalence of congenital forms is 71 per 100 000, whereas there is insufficient data to determine the prevalence of the juvenile and age-related forms. Clinical diagnosis is based on clinical findings, age of onset, family history, ophthalmological examination and slit-lamp examination. 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 cataract

(other synonyms: Genetic cataract)

General information about the disease

Mendelian cataract (MC) is a large and heterogeneous group of inherited disorders affecting the crystalline lens. Cataract is a clouding, defined as any opacity of the lens (1,2). It can be classified on the basis of age of onset:

- congenital or infantile cataract, present at birth or developing within the first years of life;
- juvenile or developmental cataract developing at least later than 1 year of life;
- presenile cataract developing before the age of 45 years;
- senile or age-related cataract developing after 45 years of age; or on the basis of affected part of the lens:
- nuclear sclerotic cataract (the most common type);
- posterior subcapsular cataract;
- cortical cataract.

Congenital cataract may be an isolated defect (70% of cases) (3) or associated with any of a large number of metabolic and genetic syndromes (1). Approximately 8-25% of all congenital cataracts are hereditary (4,5). Currently about 40 genetic loci are associated with congenital cataract and nearly 1/5 of these genes are associated with syndromic forms, although their number is constantly increasing. Congenital cataract as well as developmental cataract may be monolateral or bilateral.

Clinically all cataracts are characterized by clouded vision, need for brighter light for reading, decreased night vision, photophobia, seeing "halos" around lights, frequent changes in eyeglass or contact lens prescription, fading or yellowing of colors and most of them may be associated with strabismus.

The prevalence of congenital cataracts is estimated to be about 72 per 100 000 children, whereas insufficient data is available to determine the prevalence of juvenile and age-related cataract (6).

The diagnosis of MC is based on clinical findings, age of onset, family history, pedigree, ophthalmological examination and slit-lamp examination. It is confirmed by identification of pathogenic variants in the genes involved by molecular genetic testing.

Differential diagnosis should consider other forms of non-hereditary cataract, corneal disease, eye injury or post-traumatic lens opacities, eye tumors, medications affecting the central nervous system, glaucoma, optic nerve disease and macular disease.

While congenital cataracts affect relatively few individuals, age-related cataracts are responsible for just under half of all blindness worldwide. In these individuals, the lens is clear during childhood and remains clear until the age of 45 or 50, when gradual lens opacities begin to form. Epidemiological evidence suggests the importance of genetic factors in the pathogenesis of age-related cataract (7,8), which seems to be caused by less severe damage to the same proteins, mildly impairing their function (9). This strongly suggests an association with environmental factors contributing to the disease, although recent studies indicate specific disease-related genes (9,10). In 2001, the *twin eye study* demonstrated significant genetic influence for age-related cortical cataract, with heritability accounting for 53–58% of liability for the disease. Genetic factors were likewise found to account for approximately 48% of the risk for nuclear cataract (10).

MC has different patterns of inheritance. Variations in the following genes may be transmitted in autosomal dominant and/or recessive manner: *AGK* (OMIM gene: 610345; OMIM disease: 614691), *BFSP1* (OMIM gene: 603307; OMIM disease: 611391), *BFSP2* (OMIM gene: 603212; OMIM disease: 611597), *CHMP4B* (OMIM gene: 610897; OMIM disease: 605387), *CRYAA* (OMIM gene: 123580; OMIM disease: 604219), *CRYAB* (OMIM gene: 123590; OMIM disease: 613763), *CRYBA1* (OMIM gene: 123610; OMIM disease: 600881), *CRYBA2* (OMIM gene: 600836; OMIM disease: 115900), *CRYBA4* (OMIM gene: 123631; OMIM disease: 610425), *CRYBB1* (OMIM gene: 600929; OMIM disease: 611544), *CRYBB2* (OMIM gene: 123620; OMIM disease: 601547), *CRYBB3* (OMIM gene: 123630; OMIM disease: 609741), *CRYGC* (OMIM gene: 123680; OMIM disease: 604307), *CRYGD* (OMIM gene: 123690; OMIM disease: 115700), *CRYGS* (OMIM gene: 123730; OMIM disease: 116100), *EPHA2* (OMIM gene: 176946; OMIM disease: 116600), *EYA1* (OMIM gene: 601653; OMIM disease: 602588), *FYCO1* (OMIM gene: 607182; OMIM disease: 610019), *FOXE3* (OMIM gene: 601094; OMIM disease: 612968), *FTL* (OMIM gene: 134790; OMIM disease: 600886), *GALK1* (OMIM gene: 604313; OMIM disease: 230200), *GCNT2* (OMIM gene: 600429; OMIM disease: 116700), *GJA3* (OMIM gene: 121015; OMIM disease: 601885), *GJA8* (OMIM gene: 600897; OMIM disease: 116200), *HSF4* (OMIM gene: 602438; OMIM disease: 116800), *LEMD2* (OMIM gene: 616312; OMIM disease: 212500), *LIM2* (OMIM gene: 154045; OMIM disease: 615277), *LSS* (OMIM gene: 600909; OMIM disease: 616509), *MAF* (OMIM gene: 177075; OMIM disease: 610202), *MIP* (OMIM gene: 154050; OMIM disease: 615274), *PITX3* (OMIM gene: 602669; OMIM disease: 610623), *PAX6* (OMIM gene: 607108; OMIM disease: 106210), *SIPA1L3* (OMIM gene: 616655; OMIM disease: 616851), *SLC16A12* (OMIM gene: 611910; OMIM disease: 612018), *TDRD7* (OMIM gene: 611258; OMIM disease: 613887), *UNC45B* (OMIM gene: 611220; OMIM disease: 616279), *VIM* (OMIM gene: 193060; OMIM disease: 116300), *VSX2* (OMIM gene: 142993; OMIM disease: 610092), *WSF1* (OMIM gene: 606201; OMIM disease: 116400).

Variations in the *NHS* gene (OMIM gene: 300457; OMIM disease: 302200) have X-linked inheritance.

About ~50% of MC congenital forms are determined by variations, in lens crystallins-associated genes (*CRYAA, CRYAB, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD*), 15% of variations affects genes encoding connexins (*GJA3, GJA8*) while 10% of hereditary cataracts are determined by variations in genes encoding transcription factors (*HSF4, MAF, PITX3, PAX6*) or aquaporin (5%) (3).

Pathogenic variants may include small intragenic deletions/insertions, missense, nonsense, splicing variations. Partial or whole gene deletions/duplications are also reported for the following genes: *EYA1, PITX3, PAX6, FTL, GCNT2, AGK, VSX2, GALK1, BFSP1, NHS, WSF1.*

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 70 medical genetic laboratories in the EU, and in the GTR database, offered by about 18 medical genetic laboratories in the US.

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

Test strategy

The test involves NGS sequencing of coding exons and flanking intron sequences for detection of variations in the *AGK, BFSP1, BFSP2, CHMP4B, CRYAA, CRYAB, CRYBA1, CRYBA2, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD, CRYGS, EPHA2, EYA1, FYCO1, FOXE3, FTL, GALK1, GCNT2, GJA3, GJA8, HSF4, LEMD2, LIM2, LSS, MAF, MIP, NHS, PITX3, PAX6, SIPA1L3, SLC16A12, TDRD7, UNC45B, VIM, VSX, WFS1* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detec-

tion of duplications and deletions. Sanger sequencing is also used for family segregation studies.

The tests identify variations in known causative genes in patients suspected to have Mendelian cataracts. 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 *AGK, BFSP1, BFSP2, CHMP4B, CRYAA, CRYAB, CRYBA1, CRYBA2, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGD, CRYGS, EPHA2, EYA1, FYCO1, FOXE3, FTL, GALK1, GCNT2, GJA3, GJA8, HSF4, LEMD2, LIM2, LSS, MAF, MIP, NHS, PITX3, PAX6, SIPA1L3, SLC16A12, TDRD7, UNC45B, VIM, VSX,* and *WFS1* 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 recessive 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: A recent study on pediatric cataract demonstrated a detection rate of 58% using next generation sequencing technology (11).

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 (12)	yes
Couple risk assessment	yes

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