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Genetic testing for corneal dystrophies and other corneal Mendelian diseases

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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 corneal dystrophies and other Mendelian corneal diseases (CDs). CDs are mostly inherited in an autosomal dominant manner (autosomal recessive inheritance is rare). The overall prevalence is currently unknown. CDs are caused by mutations in the *AGBL1*, *CHST6*, *COL8A2*, *DCN*, *GSN*, *KRT12*, *KRT3*, *NLRP1*, *PAX6*, *PIKFYVE*, *PRDM5*, *SLC4A11*, *TACSTD2*, *TCF4*, *TGFBI*, *UBIAD1*, *VSX1*, *ZEB1*, and *ZNF469* genes. Clinical diagnosis is based on clinical findings, ophthalmological examination, confocal microscopy and slit-lamp biomicroscopy. The genetic test is useful for confirming diagnosis and for differential diagnosis, couple risk assessment and access to clinical trials.

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Corneal dystrophies and other Mendelian corneal diseases

(other synonyms: CDs)

General information about the disease

Corneal dystrophies (CDs) are a group of genetically and clinically heterogeneous diseases restricted to the cornea. To date more than 20 CDs have been described; they share clinical features and are typically classified on an anatomical basis into three groups:

1) anterior CDs including Reis-Bücklers dystrophy (CDRB), anterior basement membrane dystrophy (ABMD), Meesmann's epithelial dystrophy (MECD), Thiel-Behnke dystrophy (CDTB), Stocker-Holt dystrophy, Granular Corneal Dystrophy type III, Gelatinous Droplike Corneal Dystrophy and others;

2) stromal CDs including Macular Corneal Dystrophy, Granular Corneal Dystrophy types I and II (GCD), Lattice Dystrophy, Granular Dystrophy, Avellino Dystrophy, Schnyder corneal Dystrophy, Fleck dystrophy, Posterior Amorphous Corneal Dystrophy and others;

3) posterior CDs including Fuch's dystrophy spectrum (FECD), Congenital hereditary Endothelial Dystrophy types I and II, Posterior Polymorphous Dystrophies.

As clinical features vary extensively between entities, CDs should be suspected in cases of spontaneous diffuse corneal opacity, especially if bilateral, and particularly if there is a positive family history.

The prevalence of different corneal dystrophies is unknown and varies from country to country (1). MECD has been recognized in Denmark, Germany, Japan, USA, Saudi Arabia and Poland (2); GCD has been extensively studied in Denmark by Møller (3); FECD is uncommon in Saudi Arabia and in Chinese Singaporeans (4).

Diagnosis of CDs is based on clinical findings, age of onset, ophthalmological examination, confocal microscopy and slit-lamp biomicroscopy. It is confirmed by detection of pathogenic variants in the causative genes.

Differential diagnosis should consider other CDs, other disorders of the corneal epithelium, such as vapor spray keratitis, mild epithelial edema and the bleb pattern of epithelial basement membrane dystrophy, monoclonal gammopathies, lecithin-cholesterol-acyltransferase deficiency and Fabry disease.

CDs have different patterns of inheritance: autosomal dominant and autosomal recessive; in particular variations can be found in the following genes: *AGBL1* (OMIM gene: 615496; OMIM disease: 615523), *CHST6* (OMIM gene: 605294; OMIM disease: 217800), *COL8A2* (OMIM gene: 120252; OMIM disease: 136800; 609140), *DCN* (OMIM gene: 125255; OMIM disease: 610048), *GSN* (OMIM gene: 137350; OMIM disease: 105120), *KRT12* (OMIM gene: 601687; OMIM disease: 122100), *KRT3* (OMIM gene: 148043; OMIM disease: 122100), *NLRP1* (OMIM gene: 606636; OMIM disease: 617388), *PAX6* (OMIM gene: 607108; OMIM disease: 106210), *PIKFYVE* (OMIM gene: 609414; OMIM disease: 121850), *PRDM5* (OMIM gene: 614161; OMIM disease: 614170), *SLC4A11* (OMIM gene: 610206; OMIM disease: 217700), *TACSTD2* (OMIM gene: 137290; OMIM disease: 204870), *TCF4* (OMIM gene: 602272; OMIM disease: 613267), *TGFBI* (OMIM gene: 601692; OMIM disease: 607541; 121820; 121900; 122200; 608471; 608470; 602082), *UBIAD1* (OMIM gene: 611632; OMIM disease: 121800), *VSX1* (OMIM gene: 605020; OMIM disease: 148300), *ZEB1* (OMIM gene: 189909; OMIM disease: 613270; 609141), and *ZNF469* (OMIM gene: 612078; OMIM disease: 229200).

Pathogenic variants may include small intragenic deletions/insertions, splice-site, missense and nonsense variants (5-7); CDs generally have incomplete penetrance.

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

Expert centers/ Published guidelines

The test is listed in the Orphanet database and is offered by about 30 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 15 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) and "American Academy of Ophthalmology" (aao.org).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the known genes. Potentially causative variants and areas of 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 CDs. 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 *AGBL1*, *CHST6*, *COL8A2*, *DCN*, *GSN*, *KRT12*, *KRT3*, *NLRP1*, *PAX6*, *PIKFYVE*, *PRDM5*, *SLC4A11*, *TACSTD2, TCF4*, *TGFBI*, *UBIAD1*, *VSX1*, *ZEB1*, and *ZNF469* 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:

- 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%.

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 is:

- 80-100% for the forms of Corneal Dystrophy associated with the TGFBI gene (Reis-Buckler dystrophy, Thiel-Behnke's dystrophy, Granular Corneal Dystrophy, Avellino's Dystrophy, Reticular Corneal or Lattice Corneal Dystrophy) (8-10);
- 95-100% for the most common forms of superficial Corneal Dystrophy not associated with the TGFBI gene (Meesmann dystrophy, gelatinous droplike corneal dystrophy) (11-15);
- 60-100% for the most common forms of stromal Corneal Dystrophy not associated with the TGFBI gene (macular corneal dystrophy, Schnyder corneal dystrophy, congenital stromal dystrophy) (16-19);
- 2-25% for posterior forms (Fuchs dystrophy, posterior polymorphic dystrophy and congenital endothelial dystrophy) (20-22).

Clinical specificity: is estimated at approximately 99% [Author's laboratory data] (23).

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 (24)	yes
Couple risk assessment	yes

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