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Genetic testing for Stargardt macular dystrophy

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for Stargardt macular dystrophy (STGD). STGD is mostly inherited in an autosomal recessive manner and rarely in an autosomal dominant manner, with an overall prevalence of 1-5 per 10 000 live births. It is caused by variations in the *ABCA4*, *CNGB3*, *ELOVL4*, *PRPH2* and *PROM1* genes. Clinical diagnosis is based on ophthalmological examination, fluorescein angiography, electroretinography, visual field testing, optical coherence tomography and color testing. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

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Stargardt macular dystrophy

(other synonyms: Stargardt macular degeneration, juvenile macular degeneration, STGD) (Retrieved from ghr.nlm.nih.gov)

General information about the disease

Stargardt macular dystrophy (STGD) is a rare congenital group of disorders including Stargardt disease/fundus flavimaculatus and Stargardt-like macular dystrophy. Onset is typically in the first two decades of life and characterized by loss of photoreceptors and retinal pigment epithelium cells, decreased visual acuity (20/200), progressive loss of central vision, scotoma, photophobia and loss of color vision.

STGD accounts for 7% of all retinal dystrophies and its estimated prevalence is 1-5 per 10 000 live births (1).

Diagnosis of STGD is based on clinical findings, ophthalmological examination, fluorescein angiography, autofluorescence, electroretinography, visual field testing, optical coherence tomography and color testing. It is confirmed by detection of pathogenic variants in causative genes.

Differential diagnosis should consider other retinal dystrophies such as central areolar choroidal dystrophy (CACD), achromatopsia (ACHM) and cone-rod dystrophy (CORD).

STGD is most often associated with variations in *ABCA4* (OMIM gene: 601691; OMIM disease: 248200), *CNGB3* (OMIM gene: 605080; OMIM disease: 248200), *ELOVL4* (OMIM gene: 605512; OMIM disease: 600110), *PRPH2* (OMIM gene: 179605) and *PROM1* (OMIM gene: 604365; OMIM disease: 603786) genes.

Pathogenic variants may include small intragenic deletions/insertions, as well as splice-site, missense, nonsense and deep intronic variants. Partial or whole gene deletions/duplications are also reported in *ABCA4* and *PRPH2* genes.

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 25 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 *ABCA4*, *CNGB3*, *ELOVL4*, *PRPH2* and *PROM1* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in *ABCA4* and *PRPH2* genes. Sanger sequencing is also used for family segregation studies

The test identifies variations in known causative genes in patients suspected to have STGD. 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 *ABCA4*, *CNGB3*, *ELOVL4*, *PRPH2* or *PROM1* 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.

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.

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.

Risk for progeny

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 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.

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: two Italian studies suggest that a mutant allele of the *ABCA4* gene can be identified in 95.7% to 100% patients with Stargardt's disease, while biallelic variations, responsible for the phenotype, are present in 75% to 78% of patients (2,3).

Clinical specificity: is estimated at approximately 99.99% [Author's laboratory data] (4).

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 (5)	yes
Couple risk assessment	yes

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