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Genetic testing for achromatopsia

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for achromatopsia. The disease has autosomal recessive inheritance, a prevalence of 1/30000-1/50000, and is caused by mutations in the *CNGB3*, *CNGA3, GNAT2*, *PDE6C*, *ATF6* and *PDE6H* genes. Clinical diagnosis is by ophthalmological examination, color vision testing and electrophysiological testing. Genetic testing is useful for confirming diagnosis and for differential diagnosis, couple risk assessment and access to clinical trials.

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Achromatopsia

(other synonyms: complete or incomplete achromatopsia, pingelapese blindness, rod monochromatism, rod monochromacy, complete or incomplete color blindness) (1).

General information about the disease

Achromatopsia (acronym ACHM) is a rare congenital disorder characterized by reduced visual acuity (<0.2), pendular nystagmus, eccentric fixation, increased sensitivity to light (photophobia), a small central scotoma, reduced or complete loss of color discrimination and absence of cone-mediated electroretinographic amplitudes (2,3). Most individuals have complete ACHM, affecting the function of all three types of cones. Incomplete ACHM is much less frequent and has similar but generally less severe symptoms.

The estimated prevalence of ACHM is 1/30,000 (4).

The diagnosis of ACHM is based on ophthalmological examination, testing of color vision and electroretinography (ERG), which shows loss of photopic but normal scotopic response. Optical coherence tomography shows progressive disruption and/or loss of the inner/outer segment junction of photoreceptors and attenuation of retinal pigmented epithelium (RPE) in the macular region. The diagnosis is confirmed by molecular genetic analysis of the responsible genes.

Differential diagnosis should consider blue cone monochromatism (BCM), Leber congenital amaurosis, other cone dystrophies, hereditary red-green color vision defects, yellow-blue defects and cerebral ACHM.

ACHM is a heterogeneous disorder with autosomal recessive inheritance. Pathogenic variants of *CNGA3* (OMIM gene: 600053; OMIM disease: 216900), *CNGB3* (OMIM gene: 605080; OMIM disease: 262300), *GNAT2* (OMIM gene: 139340; OMIM disease 613856), *PDE6C* (OMIM gene: 600827; OMIM disease: 613093), *ATF6* (OMIM gene: 605537; OMIM disease: 616517) and *PDE6H* (OMIM gene: 601190; OMIM disease: 610024) have been reported to be causative for autosomal recessive ACHM (5,6). *CNGA3* and *CNGB3* are the major causative genes of ACHM, and account for ~20-30% and 40-50% of the cases, respectively (6,7).

Pathogenic variants may include small intragenic deletions/insertions, splice site variants, missense and nonsense variations; typically, exon or whole-gene duplications/deletions are not detected.

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

This test is found in the Orphanet database and is offered by 58 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 10 accredited medical genetic laboratories in the US.

The guidelines for clinical use of this test are described in "Clinical utility gene card" (1) and "Gene reviews" (8).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in *ATF6, CNGA3, CNGB3, GNAT2, PDE6C,* and *PDE6H* genes. Potentially causative variants and regions 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 ACHM. 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 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 biallelic pathogenic variants in *CNGB3*, *CNGA3, GNAT2*, *PDE6C*, *ATF6* or *PDE6H* 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 the 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

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 genes associated with ACHM are identified in more than 75% of cases (1).

Clinical specificity is estimated at approximately 99.99% [Author's laboratory data] (9).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for achromatopsia

b) the genetic test has diagnostic sensitivity greater than or equal to other published tests (≥75% of positive tests) (1).

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	yes
Differential diagnosis	yes
Access to clinical trial (10)	yes
Couple risk assessment	yes

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