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Genetic testing for Leber congenital amaurosis

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Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for Leber congenital amaurosis (LCA). LCA is mostly inherited in an autosomal recessive manner, rarely in an autosomal dominant manner, with an overall prevalence of 2-3/100,000 live births, and is caused by mutations in the *AIPL1*, *CEP290*, *CRB1*, *CRX*, *GDF6*, *GUCY2D*, *IFT140*, *IMPDH1*, *IQCB1*, *KCNJ13*, *LCA5*, *LRAT*, *NMNAT1*, *RD3*, *RDH12*, *RPE65*, *RPGRIP1*, *SPATA7* and *TULP1* genes. Clinical diagnosis involves ophthalmological examination and electrophysiological testing (electroretinography - ERG). The genetic test is useful for confirmation of diagnosis, differential diagnosis, couple risk assessment and access to clinical trials.

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Leber congenital amaurosis

(other synonyms: LCA)(1)

General information about the disease

Leber congenital amaurosis (acronym LCA) is a rare congenital disorder characterized by poor vision, which manifests between birth and early childhood, as well as by photophobia, abnormal pupil responses, nystagmus, high hyperopia, sharply diminished electroretinogram (ERG) and keratoconus, indicated by Franceschetti oculo-digital signs, such as eye pressing, poking, and rubbing with knuckles or fingers (1, 2). Variations in *CRB1* gene may be accompanied by specific fundus features: preservation of para-arteriolar pigmented epithelium of the retina (PPRPE) and retinal telangiectasia with exudate (also known as Coats-like vasculopathy).

The estimated prevalence of LCA is 2-3/100,000 live births and accounts for 10-18% of congenital blindness (3).

The diagnosis of LCA is based on clinical findings and confirmed by detection of pathogenic gene variants.

LCA classically presents as an isolated eye anomaly without systemic involvement. Differential diagnosis should consider disorders that range from systemic syndromes such as Senior-Løken syndrome, Joubert syndrome andsimilar, to other retinal conditions such as achromatopsia, stationary night blindness and retinitis pigmentosa with which it can easily be distinguished by typical abnormal ERG patterns.

Most often, LCA is inherited in an autosomal recessive manner and associated with mutations in the following genes: *AIPL1* (OMIM gene: 604392; OMIM disease: 604393), *CEP290* (OMIM gene: 610142; OMIM disease: 611755), *CRB1* (OMIM gene: 604210; OMIM disease: 613835), *GDF6* (OMIM gene: 601147; OMIM disease: 615360), *GUCY2D* (OMIM gene: 600179; OMIM disease: 204000), *IFT140* (OMIM gene: 614620), *IQCB1* (OMIM gene: 609237; OMIM disease: 609254), *KCNJ13* (OMIM gene: 603208; OMIM

disease: 614186), *LCA5* (OMIM gene: 611408; OMIM disease: 604537), *LRAT* (OMIM gene: 604863; OMIM disease: 613341), *NMNAT1* (OMIM gene: 608700; OMIM disease: 608553), *RD3* (OMIM gene: 180040; OMIM disease 610612), *RDH12* (OMIM gene: 608830; OMIM disease: 612712), *RPE65* (OMIM gene: 180069; OMIM disease: 204100), *RPGRIP1* (OMIM gene: 605446; OMIM disease: 613826), *SPATA7* (OMIM gene: 609868; OMIM disease: 604232) and *TULP1* (OMIM gene: 602280; OMIM disease: 613843). Rare pathogenic variants in *IMPDH1* (OMIM gene: 146690; OMIM disease: 613837) are inherited in an autosomal dominant manner (1). LCA can be also caused by heterozygous or homozygous mutation in the *CRX* gene (OMIM: 602225; OMIM disease: 613829).

Pathogenic variants may include small intragenic deletions/insertions, splice site, missense and nonsense variations. For *AIPL1*, *CEP290*, *CRB1*, *CRX*, *LCA5*, *NMNAT1*, *RDH12*, *RPE65*, *RPGRIP1* and *SPATA7* genes, partial or whole gene deletions/duplications are also commonly reported.

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 23 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 16 accredited medical genetic laboratories in the US.

The guideline for clinical use of the test is described in "Genetics home reference" (ghr.nlm.nih.gov) and "Gene reviews" (1).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the *AIPL1*, *CEP290*, *CRB1*, *CRX*, *GDF6*, *GUCY2D*, *IFT140*, *IMPDH1*, *IQCB1*, *KCNJ13*, *LCA5*, *LRAT*, *NMNAT1*, *RD3*, *RDH12*, *RPE65*, *RPGRIP1*, *SPATA7* and *TULP1* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in the *AIPL1*, *CRB1*, *CRX*, *LCA5* and *RPE65* genes. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have LCA. 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 *AIPL1, CEP290, CRB1, CRX, GDF6, GUCY2D, IFT140, IMPDH1, IQCB1, KCNJ13, LCA5, LRAT, NMNAT1, RD3, RDH12, RPE65, RPGRIP1, SPATA7* or *TULP1* 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%.

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 :In a 2013 cohort study of 56 patients with LCA, a detection rate of 70% was obtained (4). For single genes the detection rates were: *AIPL1* 3.6%, *CEP290* 5.4%, *CRB1* 3.6% (5), *CRX* 1.8%, *GUCY2D* 10.7%, *LCA5* 1.8%, *RDH12* 3.6%, *RPE65* 1.8%, *RPGRIP1* 12.5%, *TULP1* 10.7%. Variations were not detected in the *SPATA7*, *RD3*, *IMPDH1*, and *LRAT* genes. For other genes not analyzed in this study, there are single-family cases with a few, in some cases only one, identified variant (3).

Clinical specificity can be estimated at approximately 99% [Author's laboratory data] (6).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for LCA;

b) the sensitivity of the test is greater than or equal to other published tests (at least 70% of positives) (4).

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	yes
Differential diagnosis	yes
Access to clinical trial (7)	yes
Couple risk assessment	yes

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