

## Genetic testing for infantile nystagmus

Andi Abeshi<sup>1,2</sup>, Pamela Coppola<sup>3</sup>, Tommaso Beccari<sup>4</sup>, Munis Dundar<sup>5</sup>, Leonardo Colombo<sup>6</sup>  
and Matteo Bertelli<sup>2,3</sup>

### Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for infantile nystagmus (IN). Forms of IN associated with variations in *CACNA1F*, *FRMD7* and *GPR143* genes have X-linked recessive inheritance, whereas variations in *SLC38A8*, *TYR* and *TYRP1* genes have an autosomal recessive inheritance and variations in *COL11A1*, *CRYBA1* and *PAX6* genes have an autosomal dominant inheritance. The prevalence of all forms of IN is estimated to be 1 in 5000.

Clinical diagnosis is based on clinical findings, age of onset, family history, ophthalmological examination, fundoscopy, electroretinography, optical coherence tomography, slit lamp examination and visual evoked potentials. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

### Infantile nystagmus

(other synonyms: genetic nystagmus, congenital nystagmus)

### General information about the disease

Infantile nystagmus (IN) is a large heterogeneous group of inherited disorders characterized by involuntary spontaneous oscillation of the eyes, present at birth or manifesting in the first three months of life. It can manifest as an isolated disorder, mainly related to variations in the *FRMD7* gene, or as part of more complex syndromes such as albinism, oculocutaneous albinism with *TYR* and *TYRP1* variations, X-linked ocular albinism with *GPR143* variations, Chediak Higashi syndrome, achromatopsia, blue cone monochromatism, X-linked congenital stationary night blindness with *CACNA1F* variations, Stickler syndrome with *COL11A1* variations, foveal hypoplasia with *SLC38A8* and *PAX6* variations, congenital cataract with *CRYBA1* variations, Leber congenital amaurosis, Joubert syndrome, Down syndrome, Bardet Biedl syndrome and many others (1).

The prevalence of all forms of IN is estimated to be 1 in 5000 (2).

Diagnosis of IN is based on clinical findings, age of onset, family history, ophthalmological examination, fundoscopy, electroretinography, optical coherence tomography, slit lamp examination and visual evoked potentials. It is confirmed by identification of pathogenic variants of causative genes by molecular genetic testing.

Differential diagnoses may include other forms of non-hereditary IN caused by drugs, retinopathy of prematurity and infectious diseases.

IN has different patterns of inheritance. When associated with variations in the *FRMD7* (OMIM gene: 300628; OMIM disease: 310700), *GPR143* (OMIM gene: 300808; OMIM disease: 300814) and *CACNA1F* (OMIM gene: 300110; OMIM disease: 300071, 300600) genes it has X-linked recessive inheritance. Variations in the *TYR* (OMIM gene:

<sup>1</sup>MAGI Balkans, Tirana, Albania

<sup>2</sup>MAGI'S Lab, Rovereto, Italy

<sup>3</sup>MAGI Euregio, Bolzano, Italy

<sup>4</sup>Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy

<sup>5</sup>Department of Medical Genetics, Erciyes University Medical School, Kayseri, Turkey

<sup>6</sup>Department of Ophthalmology, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy

Corresponding author: M. Bertelli  
E-mail: info@assomagi.org

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606933; OMIM disease: 203100, 606952), *TYRP1* (OMIM gene: 115501; OMIM disease: 203290) and *SLC38A8* (OMIM gene: 615585; OMIM disease: 609218) genes have autosomal recessive inheritance, whereas variations in the *COL11A1* (OMIM gene: 120280; OMIM disease: 604841), *CRYBA1* (OMIM gene: 123610; OMIM disease: 600881) and *PAX6* (OMIM gene: 607108; OMIM disease: 136520, 106210) genes have an autosomal dominant inheritance.

Pathogenic variants may include small intragenic deletions/insertions, splice-site, missense or nonsense variations. For *CACNA1F*, *COL11A1*, *FRMD7*, *GPR143*, *PAX6*, *SLC38A8* and *TYR* 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, for genes with recessive autosomal/ X-linked inheritance.

## Test characteristics

### Expert centers/ Published guidelines

The test is listed in the Orphanet database and is offered by more than 89 accredited medical genetic laboratories in the EU, and in the GTR database, offered by about 82 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](http://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 the *CACNA1F*, *COL11A1*, *CRYBA1*, *FRMD7*, *GPR143*, *PAX6*, *SLC38A8*, *TYR* and *TYRP1* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in the *COL11A1*, *FRMD7*, *GPR143*, *TYR* and *PAX6* genes. Sanger sequencing is also used for family segregation studies.

The tests identify variations in known causative genes in patients suspected to have IN. 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 *CACNA1F*, *COL11A1*,

*CRYBA1*, *FRMD7*, *GPR143*, *PAX6*, *SLC38A8*, *TYR*, and *TYRP1* 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%.

Recessive X linked inheritance: 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: variations in the *FRDM7* gene are identified in 83%-94% of cases of X-linked IN (3). Clinical sensitivity can reach 98.8% for syndromic IN (4).

Clinical specificity: can be estimated at approximately 99% [Author's laboratory data] (5).

## 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 (6)	yes
Couple risk assessment	yes

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