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# Genetic testing for Usher syndrome

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## Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for Usher syndrome (USH). USH is mostly transmitted in an autosomal recessive manner and is caused by variations in the *ADGRV1, CDH23, CIB2, CLRN1, HARS, MYO7A, PCDH15, PDZD7, USH1C, USH1G, USH2A, WHRN* genes. Prevalence is estimated to be 1:30,000. Clinical diagnosis is based on audiogram, vestibular tests, visual acuity test, fundus examination, color test, optical coherence tomography and electroretinography. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

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## Usher syndrome

(other synonyms: deafness-retinitis pigmentosa syndrome, Graefe-Usher syndrome, Hallgren syndrome, retinitis pigmentosa-deafness syndrome, Usher's syndrome) (Retrieved from Genetics Home Reference)

## General information about the disease

Usher Syndrome (USH) is a rare heterogeneous genetic disorder characterized by sensorineural hearing loss and progressive visual loss related to retinitis pigmentosa. There are three known clinical forms of Usher syndrome, with overlaps between the subtypes (1): USH1 (about 40% of cases and involving severe congenital progressive hearing loss and vestibular areflexia), USH2 (about 60% of cases and involving moderate slowly progressive prelingual hearing loss, without vestibular disorders) and USH3 (<3% of cases and involving childhood onset rapidly progressive post lingual hearing loss and vestibular disorders in half of patients) (2).

Ocular findings include progressive (3,4), bilateral, symmetric degeneration of the retina, starting at the periphery and first involving rods, which leads to night blindness and tunnel vision, and then cones, which causes diurnal visual impairment (5,6).

The prevalence of USH is estimated to be 1:30,000 (retrieved from Orphanet) but may be higher. (1)

Diagnosis of USH is based on hearing parameters (audiogram showing bilateral sensorineural hear loss, mostly in the high frequencies (USH2), vestibular areflexia and altered vestibular evoked myogenic potentials (USH1, USH3)) and on ocular findings (visual acuity, fundus examination, color testing, optical coherence tomography and electroretinography). It is confirmed by detection of pathogenic gene variants.

Differential diagnosis should consider other disorders that may exhibit similar signs to USH, including Albers-Schonberg disease, Alport syndrome, Alstrom syndrome, Bardet-Biedl syndrome, Cockayne syndrome, spondyloepiphyseal dysplasia congenita,

Flynn-Aird syndrome, Friedreich ataxia, Hurler syndrome, Kearns-Sayre syndrome, Norrie syndrome, Refsum's disease and Zellweger syndrome.

USH is mostly transmitted in an autosomal recessive manner and is caused by variations in the following genes: *USH1C* (OMIM gene: 605242; OMIM disease: 276904), *PCDH15* (OMIM gene: 605514; OMIM disease: 602083, 601067), *CDH23* (OMIM gene: 605516; OMIM disease: 601067), *USH1G* (OMIM gene: 607696; OMIM disease: 606943), *CIB2* (OMIM gene: 605564; OMIM disease: 614869) or *MYO7A* (OMIM gene: 276903; OMIM disease: 276900) related to USH1; *USH2A* (OMIM gene: 608400; OMIM disease: 276901), *WHRN* (OMIM gene: 607928; OMIM disease: 611383), *ADGRV1* (OMIM gene: 602851; OMIM disease: 605472) or *PDZD7* (OMIM gene: 612971; OMIM disease: 605472) related to USH2 and *CLRN1* (OMIM gene: 606397; OMIM disease: 276902) or *HARS* (OMIM gene: 142810; OMIM disease: 614504) related to USH3. Some reports indicate digenic inheritance for *CDH23* and *PCDH15, ADGRV1* and *PDZD7*. Penetrance of USH is usually complete (5,6).

Pathogenic variants may contain small intragenic deletions/insertions, splice-site, missense and nonsense variants; exon or whole-gene duplications/deletions have also been reported in *ADGRV1, CDH23, MYO7A, PCDH15, USH1C* and *USH2A*.

## 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 26 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 21 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 "Gene reviews" (5,6).

### Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the *ADGRV1, CDH23*, *CIB2, CLRN1*, *HARS, MYO7A*, *PCDH15*, *PDZD7, USH1C*, *USH1G*, *USH2A*, *WHRN* genes. For the *USH2A* gene, we look for the deep intronic variations c. 5573-843A>G, c.8845+628C>T, c.9959-4159A>G (7) and c.7595-2144A>G (7,8). Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in *USH2A* and *PCDH15* genes. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have USH. 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 *ADGRV1, CDH23*, *CIB2, CLRN1*, *HARS, MYO7A*, *PCDH15*, *PDZD7, USH1C*, *USH1G*, *USH2A* or *WHRN* 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

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 is estimated at about 80% for USH1 and USH2 (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 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 (10) | yes |
| Couple risk assessment | yes |

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