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Genetic testing for non syndromic retinitis pigmentosa

Andi Abeshi[1,2], Alice Bruson[2], Tommaso Beccari[3], Munis Dundar[4], Fabiana D'Esposito[5,6,7] and Matteo Bertelli[2,7]

Abstract

We reviewed the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for non syndromic retinitis pigmentosa (NSRP). NSRP is determined by variations in the *ABCA4*, *AGBL5*, *ARL2BP*, *ARL6*, *BBS2*, *BEST1*, *C2orf71*, *C8orf37*, *CA4*, *CDHR1*, *CERKL*, *CLRN1*, *CNGA1*, *CNGB1*, *CRB1*, *CRX*, *DHDDS*, *EYS*, *FAM161A*, *FSCN2*, *GUCA1B*, *HGSNAT*, *IDH3B*, *IFT140*, *IFT172*, *IMPDH1*, *IMPG2*, *KIZ*, *KLHL7*, *LRAT*, *MAK*, *MERTK*, *NEK2*, *NR2E3*, *NRL*, *OFD1*, *PDE6A*, *PDE6B*, *PDE6G*, *POMGNT1*, *PRCD*, *PROM1*, *PRPF3*, *PRPF31*, *PRPF4*, *PRPF6*, *PRPF8*, *PRPH2*, *RBP3*, *RDH12*, *RGR*, *RHO*, *RLBP1*, *ROM1*, *RP1*, *RP2*, *RP9*, *RPE65*, *RPGR*, *SAG*, *SEMA4A*, *SLC7A14*, *SNRNP200*, *SPATA7*, *TOPORS*, *TTC8*, *TULP1*, *USH2A*, *ZNF408* and *ZNF513* genes. Its overall prevalence is 1 per 4000. It is mostly inherited in an autosomal recessive manner, fewer genes have autosomal dominant or X-linked recessive transmission. Clinical diagnosis is based on clinical findings, ophthalmological examination, best corrected visual acuity (BCVA), slit lamp biomicroscopy, fundus autofluorescence, electroretinography, color vision testing and optical coherence tomography. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

[1]MAGI Balkans, Tirana, Albania
[2]MAGI'S Lab, Rovereto, Italy
[3]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[4]Department of Medical Genetics, Erciyes University Medical School, Kayseri, Turkey
[5]Head and Neck Department, School of Medicine and Surgery, University of Naples "Federico II", Italy
[6]ICORG (Imperial College Ophthalmology Research Group), Western Eye Hospital, London, United Kingdom
[7]MAGI Euregio, Bolzano, Italy

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

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Non syndromic retinitis pigmentosa

(other synonyms: pigmentary retinopathy, rod-cone dystrophy, RP, tapetoretinal degeneration)(Retrieved from Orphanet, OMIM.org, Genetics Home Reference)

General information about the disease

Non syndromic retinitis pigmentosa (NSRP) is a large heterogeneous group of inherited pigmentary retinopathies affecting the retinal photoreceptors/RPE complex and leading to progressive visual loss. Typically, the primary loss would be of rod photoreceptors, followed by loss of cones, and this explains why night blindness and visual field constriction usually are the first symptoms at presentation, only later affecting the central retina with progressive visual impairment. As photoreceptor loss progresses, there is increasing rearrangement of pigment from the retinal pigment epithelium associated with intraretinal clumping of melanin, which gives the typical fundoscopic finding (bone-spikle shaped deposits).

NSRP is characterized by early progressive nyctalopia, progressive concentric restriction of the visual field (tunnel vision) and eventually, at later stages, macular involvement with severely reduced visual acuity. Other common findings may include cataract and cystoids macular oedema (1).

The prevalence of NSRP is estimated being approximately 1 in 4000 (2-6).

The diagnosis of NSRP is based on clinical findings, ophthalmological examination, best corrected visual acuity (BCVA), fundoscopy, fundus autofluorescence, electroretinography, and optical coherence tomography. It is confirmed by the identification of pathogenic variants in related genes by molecular genetic testing.

Differential diagnosis may include: syndromic RP, the most common being Usher syndrome type 1, 2 or 3 (OMIM: 276900, 276901, 276902) characterized by congenital hearing impairment and subsequent RP; other pigmentary retinopathies such as CORDs and LCA; other retinal dystrophies such as choroideremia or vitreoretinopathies and secondary pigmentary changes caused by drugs, toxins and inflammation (6).

NSRP is very variable in terms of age of onset, which may span from early infancy to after midlife (7).

NSRP may be transmitted as an autosomal recessive, dominant, or X-linked character.

The recessive form is associated with variations in the following genes: *ABCA4* (OMIM gene: 601691; OMIM disease: 601718), *AGBL5* (OMIM gene: 615900; OMIM disease: 617023), *ARL2BP* (OMIM gene: 615407; OMIM disease: 615434), *ARL6* (OMIM gene: 608845; OMIM disease: 613575), *BBS2* (OMIM gene: 606151; OMIM disease: 616562), *BEST1* (OMIM gene: 607854; OMIM disease: 613194), *C2orf71* (OMIM gene: 613425; OMIM disease: 613428), *C8orf37* (OMIM gene: 614477; OMIM disease: 614500), *CDHR1* (OMIM gene: 609502; OMIM disease: 613660), *CERKL* (OMIM gene: 608381; OMIM disease: 608380), *CLRN1* (OMIM gene: 606397; OMIM disease: 614180), *CNGA1* (OMIM gene: 123825; OMIM disease: 613756), *CNGB1* (OMIM gene: 600724; OMIM disease: 613767), *CRB1* (OMIM gene: 604210; OMIM disease: 600105), *DHDDS* (OMIM gene: 608172; OMIM disease: 613861), *EYS* (OMIM gene: 612424; OMIM disease: 602772), *FAM161A* (OMIM gene: 613596; OMIM disease: 606068), *HGSNAT* (OMIM gene: 610453; OMIM disease: 616544), *IDH3B* (OMIM gene: 604526; OMIM disease: 612572), *IFT140* (OMIM gene: 614620; OMIM disease: 266920), *IFT172* (OMIM gene: 607386; OMIM disease: 616394), *IMPG2* (OMIM gene: 607056; OMIM disease: 613581), *KIZ* (OMIM gene: 615757; OMIM disease: 615780), *LRAT* (OMIM gene: 604863; OMIM disease: 613341), *MAK* (OMIM gene: 154235; OMIM disease: 614181), *MERTK* (OMIM gene: 604705; OMIM disease: 613862), *NEK2* (OMIM gene: 604043; OMIM disease: 615565), *NR2E3* (OMIM gene: 604485; OMIM disease: 611131), *PDE6A* (OMIM gene: 180071; OMIM disease: 613810), *PDE6B* (OMIM gene: 180072; OMIM disease: 613801), *PDE6G* (OMIM gene: 180073; OMIM disease: 613582), *POMGNT1* (OMIM gene: 606822; OMIM disease: 617123), *PRCD* (OMIM gene: 610598; OMIM disease: 610599), *PROM1* (OMIM gene: 604365; OMIM disease: 612095), *RBP3* (OMIM gene: 180290; OMIM disease: 615233), *RDH12* (OMIM gene: 608830; OMIM disease: 612712), *RGR* (OMIM gene: 600342; OMIM disease: 613769), *RHO* (OMIM gene: 180380; OMIM disease: 613731), *RLBP1* (OMIM gene: 180090; OMIM disease: 136880), *ROM1* (OMIM gene: 180721; OMIM disease: 608133), *RP1* (OMIM gene: 603937; OMIM disease: 180100), *RPE65* (OMIM gene: 180069; OMIM disease: 613794), *SAG* (OMIM gene: 181031; OMIM disease: 613758), *SEMA4A* (OMIM gene: 607292; OMIM disease: 610282), *SLC7A14* (OMIM gene: 615720; OMIM disease: 615725), SPATA7 (OMIM gene: 609868; OMIM disease: 604232), *TTC8* (OMIM gene: 608132; OMIM disease: 613464), *TULP1* (OMIM gene: 602280; OMIM disease: 600132), *USH2A* (OMIM gene: 608400; OMIM disease: 613809), *ZNF408* (OMIM gene: 616454; OMIM disease: 616469) or *ZNF513* (OMIM gene: 613598; OMIM disease: 613617). It may also be inherited as an autosomal dominant character associated with variations in the following genes: *BEST1* (OMIM gene: 607854; OMIM disease: 613194), *CA4* (OMIM gene: 114760; OMIM disease: 600852), *CRX* (OMIM gene: 602225; OMIM disease: 120970), *FSCN2* (OMIM gene: 607643; OMIM disease: 607921), *GUCA1B* (OMIM gene: 602275; OMIM disease: 613827), *IMPDH1* (OMIM gene: 146690; OMIM disease: 180105), *KLHL7* (OMIM gene: 611119; OMIM disease: 612943), *NR2E3* (OMIM gene: 604485; OMIM disease: 611131), *NRL* (OMIM gene: 162080; OMIM disease: 613750), *PRPF3* (OMIM gene: 607301; OMIM disease: 601414), *PRPF31* (OMIM gene: 606419; OMIM disease: 600138), *PRPF4* (OMIM gene: 607795; OMIM disease: 615922), *PRPF6* (OMIM gene: 613979; OMIM disease: 613983), *PRPF8* (OMIM gene: 607300; OMIM disease: 600059), *PRPH2* (OMIM gene: 179605; OMIM disease: 608133), *RGR* (OMIM gene: 600342; OMIM disease: 613769), *RHO* (OMIM gene: 180380; OMIM disease: 613731), *RLBP1* (OMIM gene: 180090; OMIM disease: 136880), *ROM1* (OMIM gene: 180721; OMIM disease: 608133), *RP1* (OMIM gene: 603937; OMIM disease: 180100), *RP9* (OMIM gene: 607331; OMIM disease: 180104), *SEMA4A* (OMIM gene: 607292; OMIM disease: 610282), *SNRNP200* (OMIM gene: 601664; OMIM disease: 610359) or *TOPORS* (OMIM gene: 609507; OMIM disease: 609923). Finally, it may be transmitted as an X-linked character associated with variations in the *OFD1* (OMIM gene: 300170; OMIM disease: 300424), *RP2* (OMIM gene: 300757; OMIM disease: 312600) or *RPGR* (OMIM gene: 312610; OMIM disease: 300029) genes.

Pathogenic variants may include small intragenic deletions/insertions, splice-site, missense and nonsense variations. Partial or whole gene deletions/duplications are also reported in *ABCA4, CRB1, CRX, EYS, HGSNAT, IMPG2, MAK, MERTK, NR2E3, PDE6B, PRPF31, PRPH2, RHO, RLBP1, RP1, RP2, RPE65, RPGR, SAG, SPATA7* and *USH2A* 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 more than 37 medical genetic laboratories in the EU, and in the GTR database, offered by about 25 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 the *ABCA4*, *AGBL5*, *ARL2BP*, *ARL6, BBS2, BEST1*, *C2orf71*, *C8orf37*, *CA4*, *CDHR1*, *CERKL*, *CLRN1*, *CNGA1*, *CNGB1*, *CRB1*, *CRX*, *DHDDS*, *EYS*, *FAM161A*, *FSCN2*, *GUCA1B*, *HGSNAT*, *IDH3B*, *IFT140*, *IFT172*, *IMPDH1*, *IMPG2*, *KIZ*, *KLHL7*, *LRAT*, *MAK*, *MERTK*, *NEK2*, *NR2E3*, *NRL*, *OFD1*, *PDE6A*, *PDE6B*, *PDE6G*, *POMGNT1*, *PRCD*, *PROM1*, *PRPF3*, *PRPF31*, *PRPF4*, *PRPF6*, *PRPF8*, *PRPH2*, *RBP3*, *RDH12*, *RGR*, *RHO*, *RLBP1*, *ROM1*, *RP1*, *RP2*, *RP9*, *RPE65*, *RPGR*, *SAG*, *SEMA4A*, *SLC7A14*, *SNRNP200*, *SPATA7*, *TOPORS*, *TTC8*, *TULP1*, *USH2A*, *ZNF408* and *ZNF513* genes. For the *USH2A* and *PROM1* genes, we look for the deep intronic variations: c. 5573-843A>G, c.8845+628C>T, c.9959-4159A>G (8) and c.7595-2144A>G in *USH2A* (8, 9); c.2077+521A>G in *PROM1* (10). Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in *ABCA4, CRB1, CRX*, *EYS*, *PRPF31*, *PRPH2*, *RHO, RP1*, *RP2*, *RPE65*, *RPGR* and *USH2A* genes. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have NSRP. 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*, *AGBL5*, *ARL2BP*, *ARL6, BBS2, BEST1*, *C2orf71*, *C8orf37*, *CA4*, *CDHR1*, *CERKL*, *CLRN1*, *CNGA1*, *CNGB1*, *CRB1*, *CRX*, *DHDDS*, *EYS*, *FAM161A*, *FSCN2*, *GUCA1B*, *HGSNAT*, *IDH3B*, *IFT140*, *IFT172*, *IMPDH1*, *IMPG2*, *KIZ*, *KLHL7*, *LRAT*, *MAK*, *MERTK*, *NEK2*, *NR2E3*, *NRL*, *OFD1*, *PDE6A*, *PDE6B*, *PDE6G*, *POMGNT1*, *PRCD*, *PROM1*, *PRPF3*, *PRPF31*, *PRPF4*, *PRPF6*, *PRPF8*, *PRPH2*, *RBP3*, *RDH12*, *RGR*, *RHO*, *RLBP1*, *ROM1*, *RP1*, *RP2*, *RP9*, *RPE65*, *RPGR*, *SAG*, *SEMA4A*, *SLC7A14*, *SNRNP200*, *SPATA7*, *TOPORS*, *TTC8*, *TULP1*, *USH2A*, *ZNF408* or *ZNF513* 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

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%.

In X-linked transmission, 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: the genetic diagnosis of NSRP is currently based on NGS (next generation sequencing) technology, which in recent years has increased the detection rate of NSRP from 24% to 52%, (11) although there are differences depending on the number of genes analyzed and on patient selection. In particular, Glöckle (12) confirmed this value, with a total detection rate of 55%: 41% for autosomal dominant NSRP and 60% for autosomal recessive and sporadic NSRP. Neveling (13) reported an overall detection rate of 50% for a population of non-selected RP patients. Wang (14) reported a 37% detection rate in patients with sporadic NSRP.

Clinical specificity: is estimated at approximately 99.99% [Author's laboratory data] (15).

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 (16)	yes
Couple risk assessment	yes

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