The EuroBiotech Journal
TECHNICAL REPORT
EBTNA UTILITY GENE TEST

Genetic testing for retinitis punctata albescens/fundus albipunctatus

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

Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for retinitis punctata albescens/fundus albipunctatus (RPA/FA). RPA and FA are reported to have autosomal dominant or autosomal recessive inheritance and are associated with variations in the *PRPH2, RHO, RLBP1* and *RDH5* genes. There is insufficient data to establish their prevalence. Clinical diagnosis is based on clinical findings, ophthalmological examination, optical coherence tomography, visual field testing and undetectable or severely reduced electroretinogram amplitudes. 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]MAGI Euregio, Bolzano, Italy
[4]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[5]Department of Medical Genetics, Erciyes University Medical School, Kayseri, Turkey
[6]Head and Neck Department, School of Medicine and Surgery, University of Naples "Federico II", Italy
[7]ICORG (Imperial College Ophthalmology Research Group), Western Eye Hospital, London, United Kingdom

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

Published online: 27 October 2017
doi:10.24190/ISSN2564-615X/2017/S1.30

Retinitis punctata albescens/fundus albipunctatus

General information about the disease

Retinitis punctata albescens (RPA) is a rare inherited disease characterized by childhood onset night blindness, white retinal deposits, reduced visual acuity in the range 20/40 and areas of peripheral retinal atrophy (the macula is usually spared in early stages). In later stages, there may be atrophy of the retinal pigment epithelium, progressing to geographic atrophy of the macular pigment epithelium as the visual field becomes more constricted (1).

The prevalence of RPA is currently unknown.

Fundus albipunctatus (FA) is a rare inherited disease clinically very similar to RPA, but with normal visual acuity, normal retinal pigment epithelium and stationary night blindness (2). However, recent studies suggest that FA can progress clinically in the same way as RPA.

The prevalence of FA is also currently unknown.

Diagnosis of RPA/FA is based on clinical findings, ophthalmological examination, optical coherence tomography, visual field testing and undetectable or severely reduced electroretinogram amplitudes. It is confirmed by detection of pathogenic variants in certain genes.

Differential diagnosis for RPA/FA should mostly consider retinal dystrophies ranging from Bietti crystalline retinopathy to Bardet-Biedl syndrome, hyperoxaluria, Bothnia retinal dystrophy, "Newfoundland" CORD and retinitis pigmentosa (3).

RPA/FA are reported to have autosomal dominant or autosomal recessive inheritance.

RPA is associated with variations in the *PRPH2* (OMIM gene: 179605; OMIM disease: 136880), *RHO* (OMIM gene: 180380; OMIM disease: 136880) and *RLBP1* (OMIM gene: 180090; OMIM disease: 136880) genes (4). Variations in the *RLBP1* gene are also related to Bothnia retinal dystrophy, "Newfoundland" CORD and retinitis pigmentosa, while RPA associated with variations in the *PRPH2* and *RHO* genes can present with RP and macular dystrophies.

FA is caused almost exclusively by mutations in *RDH5* (OMIM gene: 601617; OMIM disease: 136880) gene. However, mutations in two other genes, *RLBP1* (OMIM gene: 180090; OMIM disease: 136880) and *PRPH2* (OMIM gene: 179605; OMIM disease: 136880), are also known to be associated with FA (5).

Pathogenic variants may contain small intragenic deletions/insertions, splice-site, missense and nonsense variants. For *PRPH2, RHO* and *RLBP1* 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 inheritance.

Test characteristics

Expert centers/ Published guidelines

The test is listed in the Orphanet database and is offered by about 24 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 8 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).

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns in the *PRPH2, RDH5, RHO* and *RLBP1* genes. Potentially causative variants and regions with low coverage are Sanger-sequenced. MLPA is used for detection of duplications and deletions in the *PRPH2, RHO* and *RLBP1* genes. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have RPA/FA. 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 *PRPH2, RDH5, RHO* and *RLBP1* 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: Morimura et al. identified two mutant alleles in the *RLBP1* gene in 11% of 28 patients with a clinical diagnosis of RPA (6). Katsanis et al. identified two mutant alleles of the *RLPB1* gene in one of 4 families (25%) with clinical diagnosis of FA and concluded that the RPA phenotype can be considered a clinical evolution of the FA phenotype, as was detected in elderly affected family members (4). Fishman et al. (2004) identified variations in the *RLBP1* gene in 1/3 probands with RPA (33%) (7). Dessalces et al. found variations in the *RLBP1* gene in all of seven families with autosomal recessive RPA (11 patients) (8). Currently almost 50 variations in the *RDH5* gene associated with FA are known, but in many cases they are isolated variations (identified in one or few families). It is therefore impossible to estimate total epidemiological data (5).

Clinical specificity: can be 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

References

1. Humbert G, Delettre C, Sénéchal A, Bazalgette C, Barakat A, Bazalgette C, et al. Homozygous deletion related to Alu repeats in RLBP1 causes retinitis punctata albescens. Invest Ophthalmol Vis Sci. 2006 Nov;47(11):4719-24. PubMed PMID: 17065479.
2. Dryja TP. Molecular genetics of Oguchi disease, fundus albipunctatus, and other forms of stationary night blindness: LVII Edward Jackson Memorial Lecture. Am J Ophthalmol. 2000 Nov;130(5):547-63. PubMed PMID: 11078833.
3. Wang NK, Chuang LH, Lai CC, Chou CL, Chu HY, Yeung L, et al. Multimodal fundus imaging in fundus albipunctatus with RDH5 mutation: a newly identified compound heterozygous mutation and review of the literature. Doc Ophthalmol. 2012 Aug;125(1):51-62. PubMed PMID: 22669287. Epub 2012/06/06.
4. Katsanis N, Shroyer NF, Lewis RA, Cavender JC, Al-Rajhi AA, Jabak M, Lupski JR. Fundus albipunctatus and retinitis punctata albescens in a pedigree with an R150Q mutation in RLBP1. Clin Genet. 2001 Jun;59(6):424-9. PubMed PMID: 11453974.
5. Skorczyk-Werner A, Pawłowski P, Michalczuk M, Warowicka A, Wawrocka A, Wicher K, et al. Fundus albipunctatus: review of the literature and report of a novel RDH5 gene mutation affecting the invariant tyrosine (p.Tyr175Phe). J Appl Genet. 2015 Aug;56(3):317-27. PubMed PMID: 25820994; PubMed Central PMCID: PMC4543405. Epub 2015/03/28.
6. Morimura H, Berson EL, Dryja TP. Recessive mutations in the RLBP1 gene encoding cellular retinaldehyde-binding protein in a form of retinitis punctata albescens. Invest Ophthalmol Vis Sci. 1999 Apr;40(5):1000-4. PubMed PMID: 10102299.
7. Fishman GA, Roberts MF, Derlacki DJ, Grimsby JL, Yamamoto H, Sharon D, et al. Novel mutations in the cellular retinaldehyde-binding protein gene (RLBP1) associated with retinitis punctata albescens: evidence of interfamilial genetic heterogeneity and fundus changes in heterozygotes. Arch Ophthalmol. 2004 Jan;122(1):70-5. PubMed PMID: 14718298.
8. Dessalces E, Bocquet B, Bourien J, Zanlonghi X, Verdet R, Meunier I, CP Hamel. Early-onset foveal involvement in retinitis punctata albescens with mutations in RLBP1. JAMA Ophthalmol. 2013 Oct;131(10):1314-23. PubMed PMID: 23929416.
9. Chen B, Gagnon M, Shahangian S, Anderson NL, Howerton DA, Boone JD. Good laboratory practices for molecular genetic testing for heritable diseases and conditions. MMWR Recomm Rep. 2009 Jun;58(RR-6):1-37; PubMed PMID: 19521335.
10. Stone EM, Aldave AJ, Drack AV, Maccumber MW, Sheffield VC, Traboulsi E, Weleber RG. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. Ophthalmology. 2012 Nov;119(11):2408-10. PubMed PMID: 22944025. Epub 2012/09/01.