

Molecular testing in non-syndromic retinitis pigmentosa

Disease definition : Retinitis Pigmentosa (RP) is the most common form of progressive visual degeneration of the photoreceptors (rods and cones) and/or the retinal pigment epithelium of the retina.

Frequency : RP affects approximately 1 in 5000 persons.

Main clinical symptoms : Initially loss of rod function develops, leading to night blindness followed by constriction of the peripheral visual field (tunnel vision). Eventually also central vision is affected due to loss of cone function. Early-onset RP is indistinguishable from Leber congenital amaurosis (LCA).

Inheritance : Non-syndromic RP can be inherited in an autosomal dominant (15-25 %), autosomal recessive (10-30 %), or X-linked (10-15 %) manner. Sporadic cases with unknown mode of inheritance represent 40-50 %. Some patients have digenic RP caused by one mutation in the RDS gene and another in the ROM1 gene.

Clinical diagnosis : If RP is suspected, funduscopy, visual field testing and electro-retinography should be performed.

- **Funduscopy:** bone-spicule deposits and attenuated retinal vessels are hallmarks of RP.
- **Visual field testing:** this test may show peripheral visual field restriction, ranging from ring scotoma (blind spot) in the early stages to "tunnel vision" in later stages.
- **Electroretinography (ERG):** this test may determine the functional status of the photoreceptors.

Clinical classification : RP can be subdivided in syndromic (40 %) and non-syndromic (60 %) forms. The most frequent forms of syndromic RP are Usher syndrome (prelingual hearing impairment followed by development of RP) and Bardet-Biedl syndrome.

Molecular testing : Up to now more than 48 loci with 37 nuclear genes have been shown to be implicated in non-syndromic RP. All loci have been classified as RP, followed by a number indicating the chronological order of identification of the locus. Leber congenital amaurosis (LCA) is caused by mutations in an overlapping set of genes.

- **Autosomal dominant RP:** 15 genes have been shown to cause autosomal dominant RP. The RHO gene (20-30 % of autosomal dominant mutations) encoding rhodopsin, the RDS gene (5-10 %) encoding peripherin, and the PRPF31 (5-10 %) encoding U4/U6 snRNP-associated 61-kD altogether harbour 40-50 % of all autosomal dominant mutations, and are not too large to be included in a diagnostic panel. The RP1, IMPDH1, PRPF8 genes each also contain a few % of the autosomal dominant mutations, but these genes are large, and therefore not really amenable to diagnostic tests. Frequent mutations include p.P23H and p.P347 in RHO, and p.R677X and p.Q679X in RP1. A microarray with more than 300 mutations in 13 genes (CA4, FSCN2, IMPDH1, NRL, PRPF3, PRPF31, PRPF8, RDS, RHO, ROM1, RP1, RP9, CRX) is also available for diagnostic testing of autosomal dominant RP.
- **Autosomal recessive RP:** 20 genes have been shown to cause autosomal recessive RP. However, molecular diagnosis is problematic as less than 20 % of autosomal recessive mutations are located in genes that are small enough to sequence in a diagnostic setting : these include CRB1 (5 %) encoding the Crumbs homolog 1 precursor, PDE6A and PDE6B (each 3-5 %) encoding the Rod cGMP-specific 3', 5'-cyclic phospho-diesterase alpha and beta subunit, respectively. The ABCA4 gene encoding a retina-specific ABC transporter also represents 5 % of patients, but it is rather large to sequence. The USH2A gene encoding Usher syndrome type 2A protein is with 5-10 % of mutations the gene most frequently involved in autosomal recessive RP, but its 72 exons make it a very expensive test. A microarray with more than 500 mutations in 16 genes (CERKL, CNGA1, CNGB1, MERTK, PDE6A, PDE6B, PNR, RDH12, RGR, RLBP1, SAG, TULP1, CRB1, RPE65, USH2A, USH3A) is also available for diagnostic testing of autosomal recessive RP.
- **X-linked RP:** 2 X-linked genes RPGR and RP2 have been shown to cause severe forms of RP, with most affected males showing partial or complete blindness by the age of 40. The RPRG gene encoding the RP GTPase regulator represents 70-80 % of X-linked mutations; most RPRG mutations are located in exon ORF15. Approximately 20 % of X-linked RP is due to mutations in the RP2 gene; as this gene is only 5 exons long it can be analysed easily in a diagnostic setting.
- **Sporadic cases:** Sporadic cases with unknown mode of inheritance represent 40-50 % of RP. A microarray with more than 500 mutations in 16 genes implicated in recessive RP, and a microarray with more than 300 mutations in 13 genes causing dominant RP might be the first tests to be performed. Also the genes with the

most frequent mutations that are not too large (RHO, RDS, USH2A-exon 13) might be analysed. The X-linked RPRG gene is also a good candidate in male patients.

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Databases

www.retina-international.org/sci-news/disloci.htm

<https://vpn.erasmusmc.nl/http/www.sph.uth.tmc.edu/Retnet/sum-dis.htm#A-genes>

Table 1. Different types of non-syndromic RP with the proportion of the respective gene, its size, and price indication.

Type	Specific feature	Gene	Protein	Gene contribution (%)	Number of exons (AA)	Price indication (Euro)
Dominant RP		RHO	Rhodopsin	20-30	5 exons (348 AA)	460
		RDS (PRPH2)	Peripherin	5-10	3 exons (346 AA)	550
		PRPF31	U4/U6 snRNP-associated 61-kD	5-10	14 exons (499 AA)	
		RP1	Oxygen-regulated protein 1	3- 5	27 exons (999 AA)	
		11 other genes	Various	< 3	Various	
		CA4, FSCN2, IMPDH1, NRL, PRPF3, PRPF31, PRPF8, RDS, RHO, ROM1, RP1, RP9, CRX	Various		Microarray with 341 mutations in 13 genes	600
Recessive RP	Usher syndrome	USH2A	Usher syndrome type IIa protein (Usherin precursor)	5-10	72 exons (5202 AA)	
		USH2A	Usher syndrome type IIa protein (Usherin precursor)		Exon 13	370
		CRB1	Crumbs homolog 1 precursor	5	12 exons (674 AA)	
		PDE6A	Rod cGMP-specific 3', 5'-cyclic phospho-diesterase alpha -subunit	3- 5	22 exons (860 AA)	
		PDE6B	Rod cGMP-specific 3', 5'-cyclic phospho-diesterase beta-subunit	3- 5	23 exons (855 AA)	
		ABCA4 (ABCR)	Retinal-specific ATPbinding cassette transporter	3- 5	50 exons (2309 AA)	550
		15 other genes	Various	< 3	Various	
		CERKL, CNGA1, CNGB1, MERTK, PDE6A, PDE6B, PNR, RDH12, RGR, RLBP1, SAG, TULP1, CRB1, RPE65, USH2A, USH3A	Various		Microarray with 501 mutations in 16 genes	600
X-linked RP	Ciliary dyskinesia, hearing loss, respiratory infections	RPRG	RP GTPase regulator	70- 80	19 exons (815 AA)	880
		RP2	XRP2 protein	10- 20	5 exons (350AA)	250

Figure 1. Suggested molecular testing in different types of non-syndromic RP.

