Research Article

Genetic testing in retinitis pigmentosa: detection rate and phenotype–genotype associations

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Abstract

Aims This study aimed to determine the detection rate of pathogenic DNA variants associated with retinitis pigmentosa and examine the phenotypic variability in patients with similar genotypes.

Methods Out of 49 individuals who had undergone genetic testing for retinitis pigmentosa, 34 were assessed to identify clinical presentation related to the condition. The specifics of genetic testing were also assessed, including the genetic panel used and the number of genes tested. These data were used to form a database upon which detection rate and trends in phenotype–genotype associations could be examined.

Results Of the 34 unrelated individuals, 21 were clinically diagnosed as having retinitis pigmentosa, whilst 15 were positive for a pathogenic variant associated with the disease. The corresponding detection rate was 71.4%. Unexpected trends in phenotype–genotype associations were noted.

Conclusions The significant heterogeneity present at both a phenotypic and genotypic level mean prognostic counselling for retinitis pigmentosa can be challenging. Traditional diagnostic tests must be used in conjunction with genetic testing to delineate an exact clinical diagnosis.

Introduction

Inherited retinal dystrophies represent a highly heterogenous group of disorders.[1] This, combined with the significant phenotypic overlap between similar conditions, means that the exact cause and associations between an individual's genotype and phenotype is yet to be clearly defined.[1]

This study focused on individuals diagnosed with retinitis pigmentosa, the most common cause of peripheral retinal dystrophy. Pathologically, changes occur in rod photoreceptor cells, with central vision subsequently being affected once the cone system becomes involved.[2] Systemic retinitis pigmentosa syndromes also exist, including Usher syndrome type I, where individuals are born with hearing loss, with visual disturbances becoming apparent in childhood. In contrast, types II and III of Usher syndrome are characterised by vision and hearing loss in later life.[3]

Diagnostic testing for retinitis pigmentosa includes optical coherence tomography (OCT), which reveals the presence of cystoid macular oedema.[4] In addition, fundus autofluorescence (FAF) can highlight hallmark 'bone spicules' present within the retina for the majority of patients[5] **(Figure 1)**.

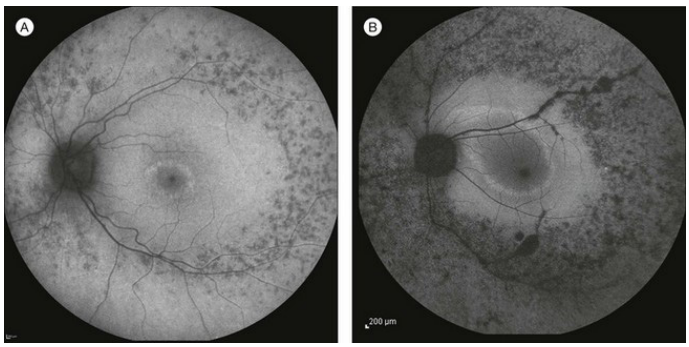

Figure 1. Fundus autofluorescence of the eye. (a) Fundus autofluorescence of the left eye in a patient with early retinitis pigmentosa. **(b)** Fundus autofluorescence of the left eye in a patient with more advanced retinitis pigmentosa. Both images show a central ring of hyper-fluorescence and a peripheral region of hypofluorescence, indicating the presence of hallmark 'bone spicules'. Reprinted from Gregory-Evans et al[6] with permission from Elsevier.

Furthermore, electrodiagnostic testing (EDT) is used to measure the electrical response of the retina to a light stimulus. Patients with retinitis pigmentosa present with reduced rod and cone amplitudes with a delay in their onset.[7]

This study aimed to determine the detection rate of pathogenic DNA variants associated with retinitis pigmentosa and examine the phenotypic variability in patients with similar genotypes.

Methods

Forty-nine patients who had undergone genetic testing for retinal dystrophies were identified by the Bristol Genetics Laboratory, and their medical notes were requested. Individuals were classed as positive if they carried a pathogenic variant known to cause retinitis pigmentosa and negative if no pathogenic variants were identified. Individuals with a variant that was felt to be insufficient alone to cause the disease, but was known to be implicated in retinal disease, were classed as 'inconclusive'. Of the 49 individuals identified, 34 individuals were included in the detection rate analysis (n=8 were excluded because their medical records were unavailable; n=2 were excluded because they were related to another individual in the study; and n=5 were excluded because their genetic test results were unavailable).

Results

Of the 34 unrelated individuals included, 21 individuals were clinically diagnosed as having retinitis pigmentosa, whilst 15 individuals were

positive for a pathogenic DNA variant that was associated with the disease. Five individuals had a negative genetic test for retinal dystrophies, and one had inconclusive results. The percentage of causative variants identified (the detection rate) was 71.4%. This was calculated by dividing the number of individuals with a positive genetic test result (15), by the total number of unrelated individuals (21).

Table 1 shows the 15 individuals who were positive for a pathogenic variant associated with retinitis pigmentosa. Individuals who were similar for a particular pathogenic variant were more likely, although not guaranteed, to develop resembling phenotypes, despite differences in their zygosity.

Table 1. Genotype of the 15 individuals who were positive for a pathogenic variant associated with retinitis pigmentosa.

Individual number	Genetic test results	Zygosity
1	*RDH12*	Compound heterozygous
2	*RDH12*	Heterozygous
	PROM1	Homozygous
	PITPNM3	Heterozygous
3	*CNGA1*	Compound heterozygous
4	*CNGA3*	Heterozygous
	CLRN1	Heterozygous
5	*RP1*	Heterozygous
	GPR179	Heterozygous
6	*TOPORS*	Heterozygous
7	*EYS*	Compound heterozygous
8	*USH2A*	Compound heterozygous
9	*USH2A*	Compound heterozygous
10	*USH2A*	Compound heterozygous
11	*USH2A*	Compound heterozygous
12	*RPGR*	Homozygous
13	*NR2E3*	Homozygous
14	*PDE6B*	Compound heterozygous
15	*RHO*	Heterozygous

All individuals were tested using the Manchester Retinal Dystrophy Panel. Panels varied from testing 105 to 176 genes.[8]

The data are colour-coded to indicate individuals who were similar for a particular pathogenic variant.

***RDH12* genotype–phenotype associations** Loss of function in the *RDH12* gene has been associated with autosomal recessive retinitis pigmentosa.[9] Individuals 1 and 2 presented at a similar age (45 and 47, respectively). Both had extensive bone spicule pigmentation bilaterally. Nonetheless, significant differences were evident on visual field testing: individual 2 had reduced visual fields concentrically, but visual fields in individual 1 were immeasurable due to little vision being maintained.

Interestingly, individual 2 was positive for two further pathogenic variants in the *PITPNM3 and PROM1* genes.

***USH2A* genotype–phenotype associations** Defective forms of the *USH2A* gene are associated with Usher syndrome and autosomal recessive retinitis pigmentosa (RP12).[10] Despite large differences in phenotypic presentation **(Table 2)**, individuals 8–11 were all clinically diagnosed with autosomal recessive retinitis pigmentosa.

Table 2. The differences in phenotypic presentation between individuals 8-11, who had a pathogenic variant of the *USH2A* gene.

Individual number	Hearing ability	Visual acuity using the Snellen Chart[a]	OCT changes	Fundal examination	Visual field changes	EDT changes
8	Normal	6/7.5 bilaterally	ND	No significant change	Bilaterally constricted	ND
9	Requires hearing aids	6/5 bilaterally	Retinal layer atrophy	Bilateral bone spicules	Bilaterally constricted	Macula dysfunction bilaterally[b]
10	Normal	6/36 bilaterally	Intra-retinal oedema and retinal layer atrophy	Bilateral bone spicules	Bilaterally constricted	Reduced rod and cone amplitudes with a delay in their onset[c]
11	Requires hearing aids	6/7.5 bilaterally	ND	Bilateral epiretinal membranes[d]	Bilaterally constricted	Reduced rod and cone amplitudes with a delay in their onset[c]

[a]A test of visual acuity that consists of a series of letters, positioned in decreasing size. A patient's visual acuity is stated as a fraction. The numerator is the distance from the chart (6) and the denominator is the last line of letters a 'normal eye' would be able to read at 6 metres. For example, 6/6 indicates normal vision i.e. the patient can read the line of letters at 6 metres that a 'normal eye' would be able to read at 6 metres. A patient with poor visual acuity e.g. 6/36 indicates they are able to read at 6 metres what a normal person could read at 36 metres.[11]

[b]Not a classical finding in retinitis pigmentosa.

[c]Classical finding in retinitis pigmentosa.

[d]Fibrous tissue that develops on the surface of the macula.

ND, no data; RP, retinitis pigmentosa.

Discussion

Retinitis pigmentosa is the most common cause of peripheral retinal dystrophy.[2] In this study, we investigated whether individuals who had similar pathogenic variants associated with the condition were more likely to develop resembling phenotypes

Two individuals had pathogenic variants of the *RDH12* gene. As mentioned, loss of function in the *RDH12* gene has been associated with autosomal recessive retinitis pigmentosa.[9] Interestingly, one of these individuals (individual 2) was also positive for pathogenic variants in the *PITPNM3* and *PROM1* genes. Köhn et al[12] discovered that mutations within *PITPNM3* caused autosomal dominant retinitis pigmentosa. Consequently, genetic testing detected a variance in inheritance that would otherwise not have been identified using clinic-based methods alone. Zhang et al[13] identified homozygosity for a mutation in the *PROM1* gene in members of a consanguineous family, presenting with retinal atrophy by the age of 40 years. Their severe retinitis pigmentosa was accompanied by macular degeneration.[13] Interestingly, the father of individual 2 had macular degeneration. It is, therefore, possible that variation within the *PROM1* gene was, in fact, accountable for retinitis pigmentosa in individual 2, rather than the *RDH12* gene. This is particularly supported by the fact that the literature has predominantly identified compound heterozygous variants within the *RDH12* gene as contributing to retinitis pigmentosa development,[14] rather than the simple heterozygous variant that was apparent in individual 2.

Four individuals were found to have pathogenic variants of the *USH2A* gene in our study. A previous study reported that Cys759Phe, a missense mutation within *USH2A*, is associated with autosomal recessive retinitis pigmentosa without hearing loss.[15] One of the individuals with a variant *USH2A* gene (individual 10) presented with this exact missense alteration and did not suffer from hearing loss;

thus, these findings suggest that clinical diagnosis was accurate. On the contrary, literature has identified the Ile371PhefsTer3 variant of the *USH2A* gene, which was observed in individual 9, as only being associated with Usher syndrome type II.[16] Interestingly, individual 9 had had hearing loss for the past 15 years, for which no specific cause had been found. This finding highlights the possibility that the clinical diagnosis of retinitis pigmentosa in individual 9 was inaccurate.

In addition, the p.Cys934Trp variant of the *USH2A* gene has been associated with both Usher syndrome type II and autosomal recessive retinitis pigmentosa.[17] Individual 11 was positive for this particular variant and had abnormal hearing tests. The possibility of Usher syndrome in individuals 9 and 11 should, therefore, not be overlooked.

Despite the aforementioned hypotheses, which suggest inaccuracies in clinical diagnosis of retinitis pigmentosa based on *USH2A* gene variants, certain studies suggest that the differences in phenotypic presentation are largely due to the multifactorial nature of *USH2A* gene expression. More specifically, Bernal et al[18] studied 28 patients with Usher Syndrome type II. They identified ten different pathogenic mutations and 17 polymorphisms in the *USH2A* gene. They further observed discordant phenotypes in sibling pairs from two unrelated families.[18] Furthermore, Liu et al[19] reported clinical differences in monozygotic twins with Usher syndrome type II and suggested that variation in the expression of the *USH2A* gene is not determined simply by genetic factors. More specifically, the environment and the effects of genetic background, including modifier genes, can all lead to an atypical presentation of Usher syndrome.[19]

Conclusion Due to the significant heterogeneity present in the individuals included in this study, at both a genotypic and phenotypic level, the results suggest that traditional clinic-based methods of assessing retinitis pigmentosa cannot always provide accurate prognostic and diagnostic information. Future focus must be placed on the development of retinitis pigmentosa-specific gene panels. This will enable both an earlier diagnosis and a more definitive characterisation of the disease.

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