Genotype and Phenotype Studies in Autosomal Dominant Retinitis Pigmentosa (adRP) of the French Canadian Founder Population

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Purpose. The French Canadian population of Quebec is a unique, well-known founder population with religious, linguistic, and geographic isolation. The genetics of retinitis pigmentosa (RP) in Quebec is not well studied thus far. The purpose of our study was to establish the genetic architecture of autosomal dominant RP (adRP) and to characterize the phenotypes associated with new adRP mutations in Quebec.

Methods. Sanger sequencing of the commonly mutated currently known adRP genes was performed in a clinically well-characterized cohort of 60 adRP French Canadian families. Phenotypes were analyzed by projected visual acuity (best corrected), Goldmann visual fields, optical coherence tomography (OCT), fundus autofluorescence (FAF), and ERG. The potential effect of the novel mutations was assessed using in silico bioinformatic tools. The pathogenicity of all variants was then confirmed by segregation analysis within the families, when available.

Results. We identified the causal mutation/gene in 24 of our adRP families, as 24 (40%) of 60 patients had adRP mutations in six known adRP genes. Eleven (46%) of these mutations were in *RHO,* four mutations (17%) were found in *SNRNP200*, three mutations (12.5%) in *PRPH2/RDS*, three mutations (12.5%) in *TOPORS*, two mutations (8%) in *PRPF31*, and one mutation (4%) in *IMPDH1*. Four mutations were novel. We identified new mutations in *RHO (p.S270I)*, *PRPF31* (p.R288W), *IMPDH1* (p.Q318H), and *TOPORS* (p.H889R); the rest were previously reported. We present the genotype-phenotype characteristics of the four novel missense mutations.

Conclusions. This is the first large screening of adRP genes in the founder population of Quebec. Our prevalence of known adRP genes is 40% in the French Canadian population, which is lower than in other adRP populations around the world, illustrating the uniqueness of the French Canadian population. Our findings are crucial in expanding the current understanding of the genotypic-phenotypic spectrum of RP and documenting the genetic architecture of our founder population.

Keywords: autosomal dominant, retinitis pigmentosa, Quebec

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous blinding retinal disease, which belongs to the large group of the inherited retinal degenerations for which 54 retinal genes have thus far been found to be mutated.[1] Retinitis pigmentosa commences with progressive night blindness due to rod photoreceptor disease, accompanied by gradual loss of peripheral visual field, followed by complete blindness due to cone photoreceptor degeneration. Retinitis pigmentosa has a prevalence of 1 in 3500 worldwide and may be inherited as an autosomal dominant (adRP), autosomal recessive (arRP), X-linked (XlRP), or digenic[2] trait. To date, there are at least 20 genes identified to cause adRP[1] and their protein products have been implicated in diverse and critical aspects of retinal photoreceptor structure and metabolism such as outer segment disc formation, the phototransduction and retinoid cycles, gene expression, transcription, mRNA processing, and others.[3]

According to Haim in 2002,[4] the adRP form has a 42.9% prevalence in all RP cases, arRP in 41%, X-linked in 22%, and the remaining 12% of cases were presumed to be as a result of nongenetic factors, non-Mendelian inheritance (for example, mitochondrial or de novo mutations), or complex inheritance.[5] The most common single gene that causes adRP is rhodopsin (*RHO*; MIM# 180380), which accounts for 8% to 10% of cases with more than 130 mutations identified so far.[6,7] According to previously published data, inosine 5′-monophosphate dehydrogenase 1 (IMPDH1; MIM# 146690) is estimated to account for 5% to 10% of cases of adRP in the United States and Europe[8]; peripherin 2 (*PRPH2/RDS*; MIM# 179605) mutations account for 3% to 9% of adRP patients of mostly European ancestry,[9–11]

Table 1. Geographic Prevalence of adRP Genes

Gene and References	Prevalence	Geographical Prevalence
Rhodopsin (*RHO*; MIM# 180380)[6,7]	≈8–%10%	Most common single gene >130 mutations identified
Inosine 5′-monophosphate dehydrogenase 1 (*IMPDH1*; MIM# 146690[8]	5%–10%	USA and Europe
Peripherin 2 (*PRPH2/RDS*; MIM# 179605)[9–11]	3%–9%	European ancestry
Retinitis pigmentosa – 1 (*RP1*; MIM# 603937)[11,12]	0%–10%	Various geographic origins
Human homolog of yeast pre-mRNA splicing gene (*PRPF31*; MIM# 606419)[13,14]	1%–8%	USA and Europe
Small nuclear ribonucleoprotein 200kDa (U5) (*SNRNP200*; MIM# 601664)[13,15]	4.2%	USA and Europe
Topoisomerase I-binding RS protein (*TOPORS*; MIM# 609507)[13,15]	1%	USA and Europe

and retinitis pigmentosa–1 (*RP1*; MIM# 603937) mutations have a prevalence of 0% to 10% from various geographic origins.[11,12] The human homolog of yeast pre-mRNA splicing gene (*PRPF31*; MIM# 606419) 1% to 8%[13,14]; small nuclear ribonucleoprotein 200 kDa (U5) (*SNRNP200*; MIM# 601664) has a prevalence at least 4.2% in the Caucasian population, and topoisomerase I-binding RS protein (*TOPORS*; MIM# 609507) with prevalence of 1% in European and US cohorts.[13,15] The rest of the adRP genes have a much lower prevalence in the adRP cases. The most common adRP causing genes with their geographic prevalence are listed in the Table 1.

The French Canadian population, where we performed our analysis, is the so-called Quebec population and consists of 7.8 million people, 80% of which are French speaking. These current 7.8 million French Canadians descend from approximately 8500 settlers who came from France starting in 1608.[16,17] The French Canadian population expanded rapidly following the British Conquest of 1759, but were relatively isolated because of religious, linguistic, and geographic barriers. During the 19th and 20th centuries, the French Canadian population was enriched by immigrants of different origins. Our cohort of 60 adRP families that we have used for our study was collected at the McGill Ocular Genetics Laboratory and Clinic (MOGL) throughout 15 years from all regions of Quebec, including very isolated areas.

In the present study, based on screening of 60 families with adRP, we report the identification of 4 novel mutations in four known adRP genes in 4 affected individuals and 11 previously reported mutations in 20 affected individuals, which makes 40% of the cohort. We also report the phenotype of the four individuals carrying these novel mutations (one patient was lost to follow-up).

Methods

Recruitment of Patients, Controls, and Phenotype Analysis

Sixty index patients from adRP families were collected in a cohort after obtaining an informed consent and explanation of the outcome of the study. Fifty-five of the families were longstanding French Canadian immigrants; 5 families were recent immigrants to Quebec. Phenotypes of each patient were analyzed using best-corrected projected Snellen visual acuity (BCVA), slit lamp examination with a full dilated fundus examination and photography, Goldmann visual fields (GFV), optical coherence tomography (OCT), fundus autofluorescence (FAF), and ERG.

DNA Sequencing and Mutation Analysis of Genomic DNA

Total genomic DNA was extracted from peripheral leukocytes in blood samples by standard salting-out procedures[18] according to manufacturer recommendation (Puregen Kit; Qiagen, Courtaboeuf, France). Polymerase chain reaction product sequencing was used to screen patient DNA for variations in the entire coding area including intron-exon junctions of adRP genes (*RHO*, *PRPH2/RDS*, *PRPF31*, *IMPDH1*, and *TOPORS*) and the most frequently mutated exons of *RP1*, *PRPF3*, *PRPF8*, and *SNRNP200*. Polymerase chain reaction amplification was performed using 50 ng of genomic DNA and BIOTAQ PCR Kit (Bioline Reagents Ltd., London, UK) in 25-μL reaction for 35 cycles.

Polymerase chain reaction products were treated with ExoSapIt (USB, Cleveland, OH, USA) and sequenced with BigDyev3.1 (Applied Biosystems Incorporated, Foster City, CA, USA) and the primers described in Supplementary Table S1. Sequence reactions were purified using Montage SEQ Cleanup Kit (Merck Millipore, Darmstadt, Germany), run on an ABI 3730 Genetic Analyzer (Applied Biosystems Incorporated, Foster City, CA, USA) and analyzed with Lasergene V8.1 (DNASTAR, Madison, WI, USA).

Identified mutations were confirmed bidirectionally and then checked in family members for segregation with disease. Novel mutations were checked in 192 control DNAs (European Collection of Cell Cultures [ECACC]).

All novel mutations were analyzed using four protein structure prediction programs: PolyPhen-2, Sorting Intolerant From Tolerant (SIFT), pMUT, and PSIPRED. PolyPhen-2 uses sequence- and structure-based predictive algorithms. The method generates a different scale of reported scores, where a score of 0 to 0.2 is considered "benign," 0.2 to 0.85 is "possibly damaging," and 0.85 to 1.0 is "probably damaging."[19] The SIFT tool, which generates multiple alignments of the sequence over different species to look at the conserved sequences of a gene, assesses the conserved amino acid positions and analyzes the effect of missense changes on the conserved structure of proteins over the course of evolution. The SIFT tool assigns a score to the mutations, and the score of less than 0.05 is considered potentially damaging.[20] The pMUT is based on the use of different kinds of sequence information to label mutations, and neural networks (NNs) to process this information. Neural network values from 0 to 1. > 0.5 is predicted as a disease-associated mutation.[21] The PSIPRED is a simple and accurate secondary structure prediction method, incorporating two feed-forward NNs that perform an analysis on output obtained from Position Specific Iterated-BLAST (PSI-BLAST).[22] For PSIPRED analysis, any predicted changes in protein secondary structure were considered to be damaging mutations.

Results

Identification of adRP Mutations

A cohort of 60 index patients with adRP collected in the French Canadian population underwent direct sequencing of

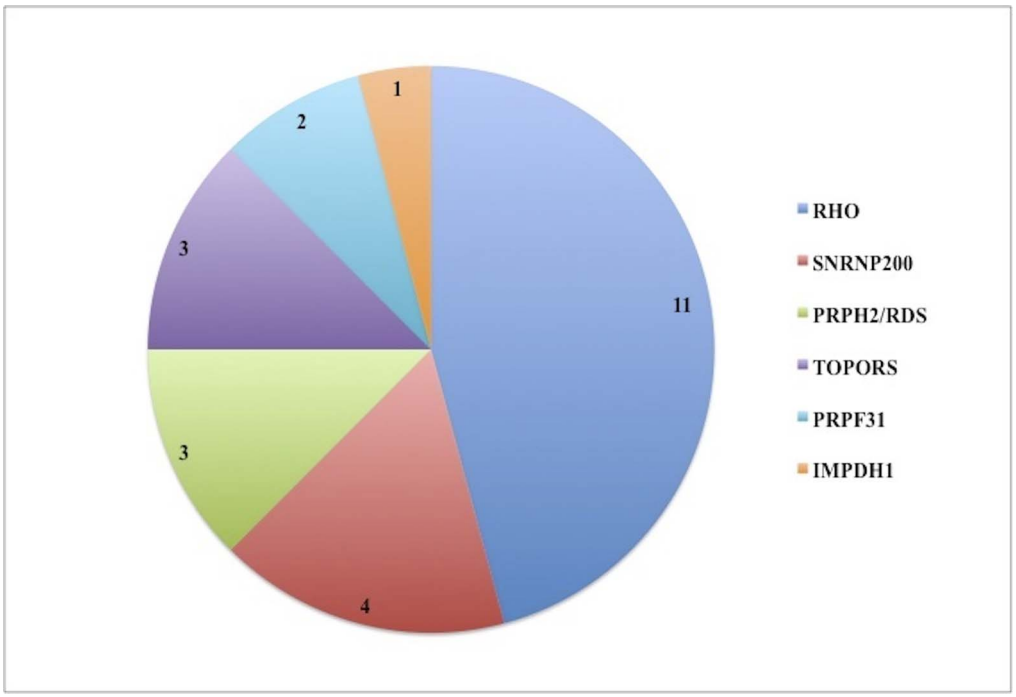

Figure 1. Frequency (number of patients out of 60) of adRP patients found in the French Canadian cohort. Gene abbreviations: rhodopsin (*RHO*); small nuclear ribonucleoprotein 200 kDa (U5) (*SNRNP200*); peripherin 2 (*PRPH2/RDS*); the human homolog of yeast pre-mRNA splicing gene (*PRPF31*); topoisomerase I-binding RS protein (*TOPORS*), and inosine 5′-monophosphate dehydrogenase 1 (IMPDH1). The analysis identified mutation in 40% (24/60) of the French Canadian adRP cohort of 60 families. Mutations have yet to be identified in the remaining 60%.

the coding areas and flanking sequences as described previously. We then identified the causal mutation/gene in 24 adRP families, as 24 (40%) of 60 patients had adRP mutations in six known adRP genes. Eleven (46%) of these mutations were in *RHO*, four mutations (17%) were found in *SNRNP200*, three mutations (12.5%) in *PRPH2/RDS*, three mutations (12.5%) in *TOPORS*, two mutations (8%) in *PRPF31*, and one mutation (4%) in *IMPDH1*. We identified five novel mutations. All identified mutations are missense mutations and are present in a heterozygous state according to the autosomal dominant status of the family they belong to (Fig. 1; Table 2). We also present the phenotypic characteristics of three novel missense mutations identified in *PRPF31* (p.R288W), *IMPDH1* (p.Q318H), and *TOPORS* (p.H889R).

Novel Mutations and Their Phenotypes

PRPF31 c.862C>T (p.R288W). The patient with this PRPF31 mutation was a 68-year-old woman who reported 2 years of blurry vision OU. Her BCVA was 20/40 in each eye and had an unremarkable anterior segment examination. A pericentral ring scotoma was seen on Goldman visual field (not shown). Her ERG showed 75% and 50% amplitude preservation in rods and cones, respectively (not shown). As per the mutation prediction scoring systems, *PRPF31 c.862C>T* was also considered probably damaging, deleterious, pathological, and associated with secondary protein changes as per PolyPhen-2, SIFT, pMUT, and PSIPRED, respectively (Table 3).

The patient fundus photos of the OD showed central macular mottling with atrophic changes as well as choroidal

Table 2. Mutation Spectrum of adRP in the French Canadian Cohort

Gene	Nucleotide Change	Predicted Effect	Location in the Gene	Novel/Known Change	Type of Change	Patient Frequency	Control Frequency
RHO	c.403C>G	p.R135G	Exon 2	Known	Heterozygous	2/60.	0/192
RHO	c.809G>T	p.S270I	Exon 4	Novel	Heterozygous	1/60.	0/192
RHO	c.1031A>C	p.Q344P	Exon 5	Known	Heterozygous	1/60.	0/192
RHO	c.151G>C	p.G51R	Exon 1	Known	Heterozygous	3/60.	0/192
RHO	c.541G>A	p.E181K	Exon 3	Known	Heterozygous	2/60.	0/192
RHO	c.553T>C	p.C185R	Exon 3	Known	Heterozygous	2/60.	0/192
SNRNP200	c.2122G>A	p.V708I	Exon 16	Known	Heterozygous	1/60.	0/192
SNRNP200	c.2041G>T	p.R681C	Exon 16	Known	Heterozygous	1/60.	0/192
SNRNP200	c.3260C>T	p.S1087L	Exon 25	Known	Heterozygous	2/60.	0/192
PRPH2/RDS	c.554T>C	p.L185P	Exon 1	Known	Heterozygous	3/60.	0/192
TOPORS	c.2666A>C	p.H889R	Exon 3	Novel	Heterozygous	1/60.	0/192
TOPORS	c.2474_2475insA	p.Y825X	Exon 3	Known	Heterozygous	2/60.	0/192
PRPF31	54618847delC		Exon 1	Known	Heterozygous	1/60.	0/192
PRPF31	c.862C>T	p.R288W	Exon 9	Novel	Heterozygous	1/60.	0/192
IMPDH1	c.954G>C	p.Q318H	Exon 9	Novel	Heterozygous	1/60.	0/192

Table 3. Mutation Prediction for Identified Novel adRP Mutations in the French Canadian Cohort

		PolyPhen-2*		SIFT†		pMUT‡			PSIPRED§
Gene Mutation	MOGL	Prediction	Human var Score	Prediction	Tolerance Index	NN Output	Reliability	Prediction	Protein Secondary Structure Change
RHO c.809G>T (p.S270I)	1637	Benign	0.07	Tolerant	0.32	0.8429	6	Pathological	Yes
TOPORS c.2666A>C (p.H889R)	355	PRD	0.993	Deleterious	0	0.4718	0	Neutral	Yes
PRPF31 c.862C>T (p.R288W)	1648	PRD	1	Deleterious	0.01	0.976	9	Pathological	Yes
IMPDH1 c.954G>C (p.Q318H)	334/2194	POS	0.772	Tolerant	0.07	0.2503	4	Neutral	Yes/No

* PolyPhen-2 appraises mutations qualitatively as benign, possibly damaging (POS) or probably damaging (PRD) based on the model's false-positive rate.[19,30]

† The SIFT results are reported to be tolerant if tolerance index ≥ 0.05 or deleterious if tolerance index < 0.05.[20,31]

‡ The pMUT is based on the use of different kinds of sequence information to label mutations, and NNs to process this information. NN = neural network values from 0 to 1. > 0.5 is predicted as a disease-associated mutation. Reliability = values 0–9. > 5 is the best prediction.[21,32]

§ The PSIPRED was used for secondary structure prediction, and the number of the resulting alterations is given in the table. For PSIPRED analysis, any predicted changes in protein secondary structure were considered to be damaging mutations.[22,33]

sclerosis (Figs. 2A–C). The FAF of the OD showed pinpoint hyperfluorescent lesions mainly concentrated nasal to the fovea (Fig. 2D). The OCT of the OD showed severe retinal changes including pigment deposition in the outer retinal layers that appear grossly distorted as well as choroidal cystic changes (Fig. 2E). Note that the mutation was found in 1 of 97,470 individuals in a heterozygous state.

TOPORS c.2666A>C (p.H889R). At the last time of examination, the patient with this TOPORS mutation was a 50-year-old woman who reported nyctalopia since the age of 12 years. She then reported a progressive constriction of her peripheral vision. Her BCVA was 20/40 OD and 20/50 OS. The ERG showed more than 95% loss in the rod amplitude compared to normal (data not presented). Based on the mutation prediction scores, our *TOPORS c.2666A>C* mutation was classified as probably damaging, deleterious, neutral and associated with secondary protein changes as per PolyPhen-2, SIFT, pMUT and PSIPRED, respectively (Table 3).

The fundus photos show a pale, blurred disc with peripapillary and peripheral bone spicules, arteriolar narrowing as well as foveal atrophy extending to the perifoveal area (Figs. 3A–C). The FAF delineates the maculopathy to a combination of a hypofluorescent foveal area surrounded by a hyperfluorescent perifoveal ring. The same refractile hyperfluorescent pinpoint lesions seen in Figure 2D can also be appreciated on Figure 3D but to a lower extent. The OCT of the patient showed a temporal schisis limited to the outer retinal layers as well as two cystic-like lesions centrally at the level of the umbo and at the choroidal level (Fig. 3E). Note that the mutation was found in 5 of 121,378 individuals (likely to be a polymorphism).

IMPDH1 c.954G>C (p.Q318H). This IMPDH1 mutation was discovered in a 49-year-old man who reported decreased peripheral vision loss, color vision fluctuation, progressively worsening nyctalopia, and central vision decrease since early childhood. His BCVA at examination was 20/400 and 20/200, OD and OS, respectively. The PolyPhen-2, SIFT, pMUT, and PSIPRED classified *IMPDH1 c.954G>C* as possibly damaging, tolerant, neutral, and undetermined associated with secondary protein changes, respectively (Table 3).

The posterior pole of the OD is remarkable for classic bone spicules, severe fundus and optic nerve palor, atrophic disc, attenuated and straightened blood vessels, and a characteristic "Bull's eye" maculopathy with extensive concentric areas of clumped hyperpigmentation (Figs. 4A–C). The maculopathy can be further appreciated on the FAF photo, which shows central hypopigmented confluent islands surrounded by a hyperpigmented crescent (Fig. 4D). Multiple diffuse parafoveal hypopigmented lesions also can be appreciated on the FAF. The OCT is remarkable for the following: severe foveal "dipping," outer retinal tissue thinning, and cystic changes. The choroidal layer contains diffuse cystic-like lesions (Fig. 4E).

Discussion

In this work we present, for the first time, a large screening of RP in an important founder population: the French Canadian population of Quebec. We screened 60 Quebec adRP families by direct Sanger sequencing of nine currently known hot spots of adRP genes (five genes in total and four genes exons hotspots). Among the 60 screened families, we were able to "settle" at the molecular level 24 patients (~40%), while surprisingly we did not find adRP mutations in the remaining 60% of families. Mutations in the 60% of Quebec adRP patients likely reside in new, to be identified genes and in locations in known genes (e.g., exons, introns, promotors) not captured in this study. We are currently testing both hypotheses. Overall, our analysis is within the previously reported estimated range of adRP mutations among adRP patients (14%–70%[23]). Blanco-Kelly et al.[24] studied 139 Spanish families with adRP and identified mutations in approximately 14% (20 families) of their cohort. However, Daiger et al.,[25] in their 2014 report, were able to identify adRP causal mutations in 70% of their 250 screened families, which is almost double our rate. We believe that the difference is mainly because Daiger et al.[25] investigated a US outbred cohort, whereas we studied a French Canadian founder cohort. Similarly, Sullivan et al.[11] found adRP causal mutations in approximately 54% of their 200 American families of European origin. Again their cohort has a higher rate of mutation detection (1.4 times the one we found).

We identified 24 causal mutations in six known adRP genes. Among the 24 identified mutations, 4 are novel (Table 3). Eleven (46%) were in *RHO,* four mutations (17%) were found in *SNRNP200*, three mutations (12.5%) in *PRPH2/RDS*, three mutations (12.5%) in *TOPORS*, two mutations (8%) in *PRPF31*, and one mutation (4%) in *IMPDH1* (Fig. 1; Table 2).

Our understanding of RP is limited to a total of 56 genes (20 in adRP), each having crucial physiological functionality related to photoreceptor function, anatomy, and/or survival. Of all RP cases, adRP represents approximately 30% to 40%.[23]

The gene with the highest mutation prevalence in our Quebec cohort is rhodopsin with 18.3% (11 of 60 screened, Fig. 1; Table 2) of mutations, confirming its significant involvement in the adRP pathogenesis in the French Canadian

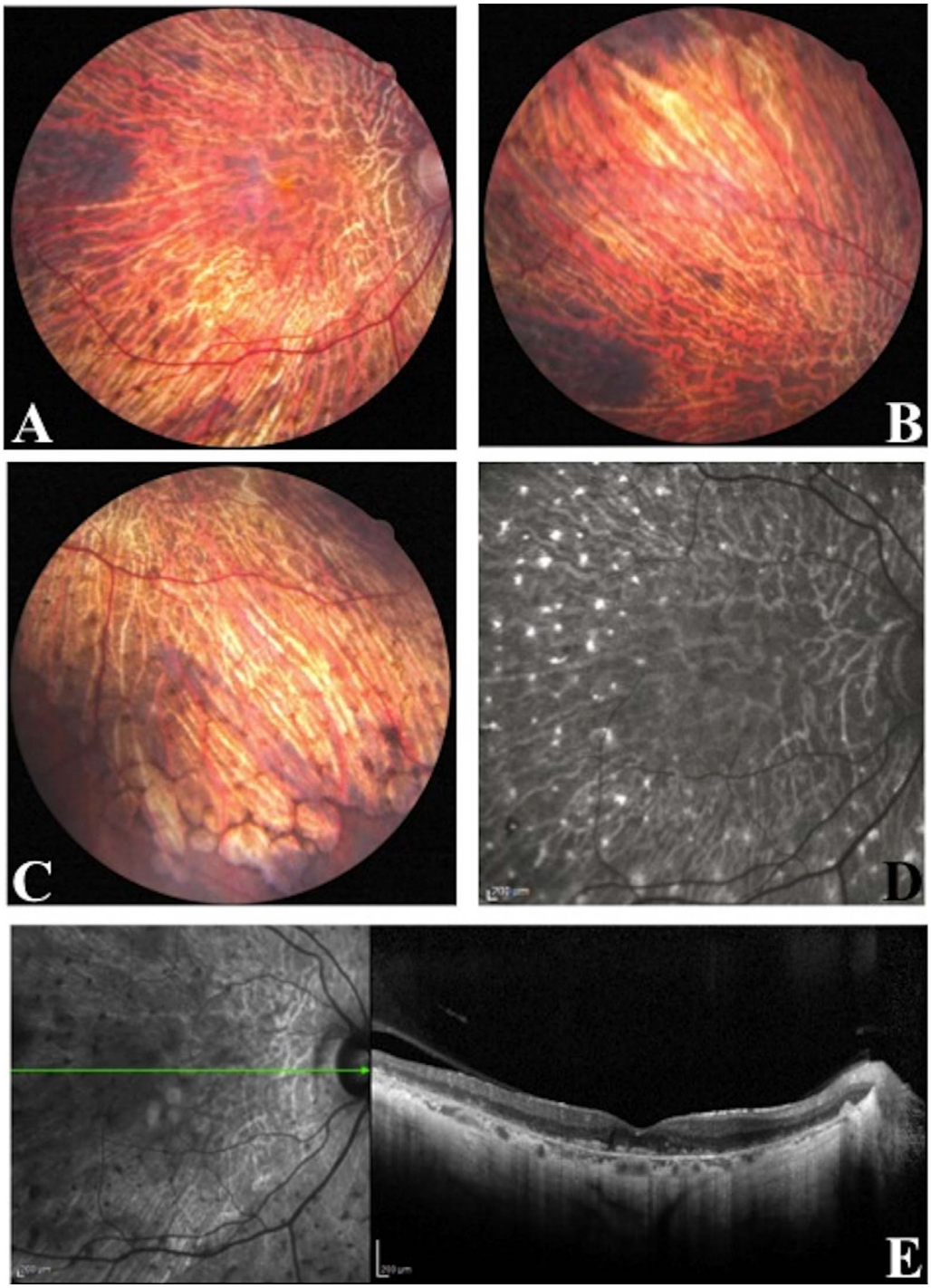

FIGURE 2. Posterior pole findings of a 68-year-old woman with *PRPF31 c.862C>T (p.R288W)* mutation. The patient reported 2 years of blurry vision OU and her BCVA was 20/40 in OU. (**A–C**) Photos of the OD fundus showing a central macular modeling with atrophic changes as well as choroidal sclerosis. (**D**) The OD FAF showing pinpoint hyperfluoresent lesions mainly concentrated nasal to the fovea. (**E**) The OD OCT showing severe retinal changes including pigment deposition in the outer retinal layers that appear grossly distorted as well as choroidal cystic changes.

population. This mutation frequency is in the known range of *RHO* mutations in other countries and cultures.[14] Studies range from 0% in India, to European countries (16%–20%) to the United States (30%) and the United Kingdom (50%). Interestingly, Dryja et al.[26] reported an approximately 30% mutation rate for rhodopsin in Americans of European origin, particularly due to the founder mutation RHO Pro23His, which had a prevalence of roughly 12%. The *SNRNP200* gene was the second most common causal gene with approximately 6.7% of all mutations (4 of 60). Both *PRPH2/RDS* and *TOPORS* accounted for 5% of all mutations (3 of 60). Of all reported mutations in the French Population of Quebec, *PRPF31* and *IMPDH1* represented 1.7% (1 of 60).

In previously reported adRP mutations, *RHO* was both the most common single causal gene with approximately 8% to 10% of all adRP mutations and the most geographically spread

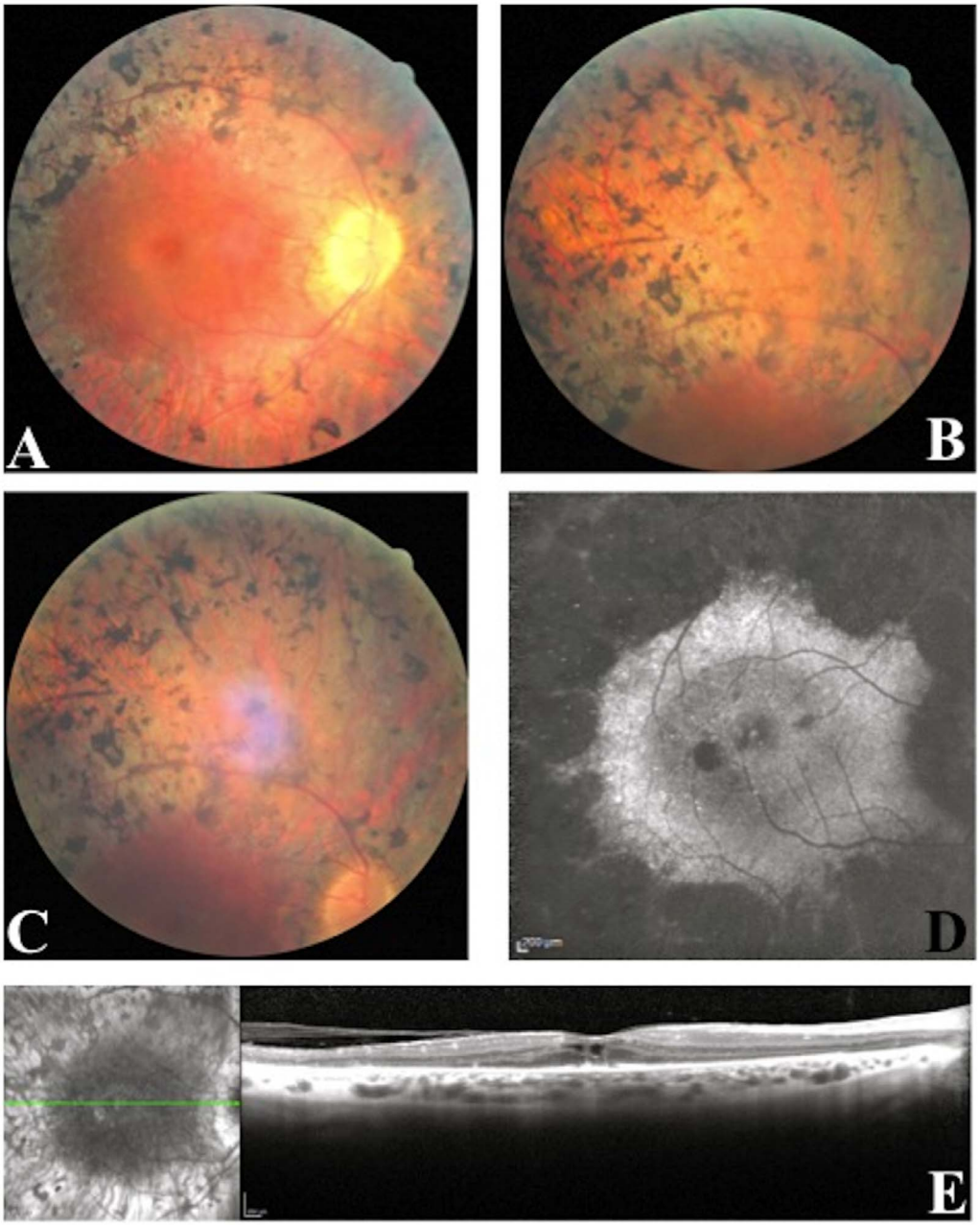

Figure 3. Posterior pole findings of a 50-year-old woman with *TOPORS c.2666A>C (p.H889R)* mutation. The patient reported nyctalpia since the age of 12 years. Her BCVA was 20/40 and 20/50, OD and OS, respectively. (**A–C**) Photos of OD fundus showing a pale, blurred disc with peripapillary and peripheral bone spicules, and arterial narrowing as foveal atrophy extending perifoveally. (**D**) An OD FAF delineating the maculopathy to a combination of a hypopigmented fovea surrounded by a hyperpigmented zone. Refractile hyperpigmented pinpoint lesions can also be appreciated. (**E**) An OD OCT showing a temporal schisis limited to the outer retinal layers as well as two cystic-like lesions centrally at the level of the umbo and at the choroidal level.

out gene with mutations found mainly in Europe, North America, and China[6,7,24,27] (Table 1 summarizes the previous adRP mutations). The *IMPDH1* mutation, which accounts for approximately 5% to 10% of all adRP mutations, was found mainly mutated in Europe and the United States.[8] Mutations in *PRPF31*, *SNRNP200*, and *TOPORS* were all also previously reported to occur mainly in Europe and the United States and represented approximately 1% to 8%, 4%, and 1%, respectively.[13–15] Sato et al.[28] reported the prevalence of *PRPF31* in Japanese patients to be approximately 3%. In 2010, Audo et al.[14] estimated the prevalence of *PRPF31* to be close to 6.7% in French adRP. The same authors found that Pro347Leu is the most common mutation in *RHO,* which was the most common casual gene in French adRP patients, representing 16.5%.[29] The *PRPH2*, which was mainly present in population of European ancestry, accounted for up to 9% of all adRP mutations.[9–11]

Of interest for population genetics is the fact that none of our identified adRP mutations and none of the reported mutations could be directly traced back to the 1608 French settlers. We expected that because the Quebec population is directly and ancestrally related to Old World France (especially Bretagne), we would find adRP mutations in common. In other words, the previously reported adRP causal mutations in Europe and North America were not, for the most part,

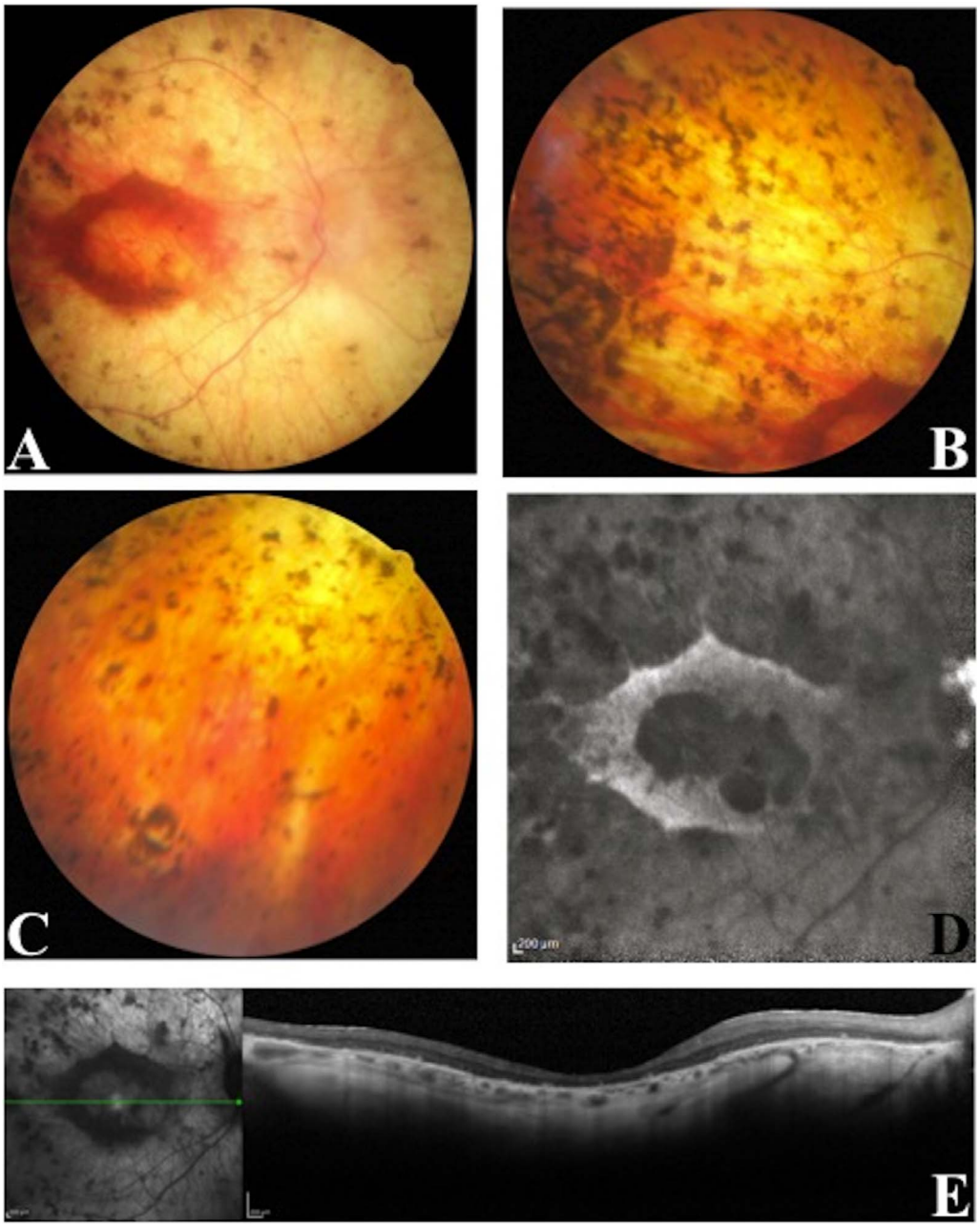

FIGURE 4. Posterior pole findings of a 49-year-old man with *IMPDH1 c.954G>C (p.Q318H)* mutation. The patient reported decreased peripheral vision loss, color vision fluctuation, progressively worsening nyctalopia, and central vision decrease since early childhood. His BCVA at examination was 20/400 and 20/200, OD and OS, respectively. (**A–C**) Photos of OD fundus showing classic bone spicules, severe fundus and optic nerve palor, atrophic disc, attenuated and straightened blood vessels, as well as a characteristic "Bull's eye" maculopathy with extensive concentric areas of clumped hyperpigmentation. (**D**) An OD FAF showing a maculopathy characterized by central hypopigemented confluent islands surrounded by a hyperpigmented crescent. (**E**) An OD OCT showing severe foveal "dipping," outer retinal tissue thinning, and macular and choroidal cystic changes.

specifically found in French families from France. The most common French mutation in adRP, which is *RHO c.1040C>T (p.Pro347Leu)*,[29] does not appear in our Quebec cohort. Vice versa, our most common mutation in *RHO* does not appear in France (Table 2). However, one Rho mutation, *RHO c.403C>G (p.Arg135Gly)*, was previously reported by Audo et al.[29] with a single base-pair difference (*c.403C>T (p.Arg135Trp)*); we believe that this can result from a hypermutable site. This finding suggests that most mutations arose after the migration and confirms the uniqueness of the founder effect on the French Canadian population of Quebec. However, based on the current findings, we still cannot exclude the fact that some of the previously European reported adRP mutations might actually have French origins.

Most patients reported progressive constriction of their peripheral vision in addition to nyctalopia. Although most patients' phenotypes exhibited classic RP fundus findings, including bone spicules, attenuated retinal vessels, and optic nerve pallor, those with novel mutations were additionally characterized by atypical bull's eye maculopathies, patchy perimacular lacunar hyperpigmentations, and macular and choroidal cystic changes, which are unusual for adRP-related pathogenesis (Figs. 2–4). Most patients' visual function was associated with decrease in ERG amplitude and perceivable

visual field changes. The mutations in *RHO*, except *p.S270I* and *PRPF31* were found to be both deleterious and pathological as per PolyPhen-2 and pMUT, respectively (Table 3), explaining the relative severity of their respective phenotypes. The other novel mutations in *TOPORS* (p.H889R) and *IMPDH1* (p.Q318H) were reported as neutral, based on pMUT; however, showed a variable spectrum of phenotypic manifestations.

The heterogeneous aspect of RP is clearly seen in the present study, as some patients presented with very severe symptoms and visual functionality, and had fundus findings that are relatively out of proportion to the severity of their symptomatology. This aspect is certainly related to the type of mutations, its loci, severity, and the pathophysiological function of the causal gene involved.

At this point, it is still difficult to provide a comprehensive map of genotype-phenotype correlations in adRP based on the data we collected. The genetics of adRP, and RP, in general, are far from being fully understood and, hence, revealed. There are likely several causal mechanisms that eventually lead to apoptotic pathways involved in the photoreceptor loss. There is still a need for significant research in both molecular pathways and its related clinical correlations to fully understand RP and provide potential treatment solutions. However, our findings in the present study are crucial in expanding the current understanding of the genotypic-phenotypic spectrum of RP and documenting the genetic architecture of a founder population.

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