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Novel *C2orf71* mutations account for ~1% of cases in a large French arRP cohort

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Novel *C2orf71* mutations account for ~1% of cases in a large French arRP cohort



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ABSTRACT: Autosomal-recessive retinitis pigmentosa (arRP) is a genetically heterogeneous group of disorders to which a novel gene, *C2orf71*, was recently associated. The purpose of our study was to establish the prevalence and nature of *C2orf71* mutations in a clinically well-characterized cohort of 345 sporadic and arRP French cases. Direct sequencing of *C2orf71* was performed in 209 subjects for whom mutations had previously been excluded by microarray technology and direct sequencing of *EYS*. Putative pathogenicity of the identified variants was evaluated through co-segregation analysis, screening of more than 188 control chromosomes and prediction programs. We identified two patients compound heterozygous for mutations predicted to lead to a premature stop codon, 3 of which are novel. In addition, 3 patients carried a single variant of likely pathogenicity. Furthermore a large number of novel putative non-disease causing variants were identified, highlighting the extremely polymorphic nature of *C2orf71*. To our knowledge, our study provides the first large scale screening of *C2orf71* in a French arRP cohort through direct sequencing and suggests that it would account for approximately 1% of arRP cases. ©2010 Wiley-Liss, Inc.

KEY WORDS: Rod-cone dystrophies, RP, *C2orf71*.

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INTRODUCTION

Rod-cone dystrophy also known as retinitis pigmentosa (RP) is a heterogeneous group of inherited disorders in most cases primarily affecting rods with secondary cone degeneration. It is the most common inherited form of potentially severe retinal degeneration and blindness, with a frequency of about 1 in 4000 births and more than 1 million individuals affected worldwide (Hartong et al. 2006). Patients initially complain of night blindness due to rod dysfunction followed by progressive visual field constriction, abnormal color vision and eventually loss of central vision. Inheritance of RP can be either autosomal recessive (ar), autosomal dominant (ad) or X-linked (xl) with also rare cases of mitochondrial transmission. Recessive and isolated cases account for 50-60% of all forms of RP (Hartong et al. 2006). So far, 32 genes have been implicated in arRP and 3 additional loci have been mapped (<http://www.sph.uth.tmc.edu/Retnet>). Most of the identified genes accounted for only 1-2% of cases each, besides *EYS* (MIM# 612424) and *USH2A* (MIM# 608400), which were found to account for up to 15.9% and up to 23% of isolated arRP respectively (Abd El-Aziz et al. 2008; Audo et al.; Bandah-Rozenfeld et al.; Barragan et al.; Littink et al. 2010) and (Rivolta et al. 2000; Bernal et al. 2003; McGee et al. 2010). Recently, the prevalence of loss-of-function mutations in *FAM161A* (MIM#613596) was found to be ~12% in arRP patients from Israel and Palestinian territories (Bandah-Rozenfeld et al. 2010b). However, in a similar study the prevalence of *FAM161A*-mutations in arRP patients from Germany was 2%-3% (Langmann et al. 2010). Future prevalence studies will show if *FAM161A* can be also considered as a major gene in other arRP populations.

Two articles in the same issue of the American Journal of Human Genetics report findings on a new causative gene, *C2orf71* (MIM#613425), for arRP using a homozygosity mapping approach on consanguineous and non-consanguineous families (Collin et al. 2010; Nishimura et al. 2010). *C2orf71* (Chromosome 2 Open reading Frame 71) harbors 2 exons and encodes for a 1288 amino-acid protein. This protein is highly expressed in retina (Collin et al. 2010; Nishimura et al. 2010) and seems to play a role in primary cilia of the outer segment (Nishimura et al. 2010), although its precise function remains to be elucidated. Bioinformatic analysis predicts a proline-rich motif at the C-terminus and functional studies suggest post-translational lipid modifications at the second and third amino acid that may be important for the sorting and function of the protein (Nishimura et al. 2010).

To assess the prevalence of *C2orf71* pathogenicity, we screened 209 patients of different ethnic origins from a French cohort of 345 arRP who had previously been excluded for known pathogenic variants by applying a commercially available microarray (ASPER Ophthalmics, Tartu, Estonia) and by direct sequencing of *EYS* (Audo et al. 2010).

PATIENTS AND METHODS

Three hundred forty five index patients with a presumed diagnosis of simplex or arRP were ascertained. Informed consent was obtained from each patient and unaffected family member after explanation of the study and its potential outcome. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Each patient underwent full ophthalmic examination as described earlier (Audo et al. 2010). Total genomic DNA was extracted from peripheral blood leukocytes according to manufacturer recommendation (Puregen Kit, Qiagen, Courtaboeuf, France). Prior to *C2orf71* mutation screening, the DNA of all index patients was analyzed for known arRP mutations by microarray analysis on a commercially available microarray (ASPER Ophthalmics, Tartu, Estonia). Samples, which were excluded for known variants were further excluded for mutations in the coding exons and flanking intronic regions of *EYS* by direct sequencing (Audo et al. 2010). The remaining 209 samples were further investigated for variants in the coding exons and flanking intronic regions of *C2orf71* by PCR and direct sequencing. The 2 coding exons, and flanking intronic regions of *C2orf71* were PCR amplified in 11 fragments (*C2orf71* RefSeq NM_001029883.1) using oligonucleotides reported in Supplementary Table 1, a commercially available polymerase (HotFire, Solis Biodyne, Tartu, Estonia), 2 mM MgCl₂ at an annealing temperature of 60°C for 1 minute. PCR products were enzymatically purified (ExoSAP-IT, USB Corporation, Cleveland, Ohio, USA purchased from GE Healthcare, Orsay, France) sequenced and investigated as previously reported (Audo et al. 2010). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. The correct nomenclature for each change was checked applying Mutalyzer (<http://www.lovd.nl/mutalyzer/>). PolyPhen (Polymorphism Phenotyping, <http://genetics.bwh.harvard.edu/pph/>; <http://genetics.bwh.harvard.edu/pph2/>) (Ramensky et al. 2002; Adzhubei et al. 2010), and SIFT (Sorting Intolerant From Tolerant; <http://blocks.fhcrc.org/sift/SIFT.html>) (Ng and Henikoff

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2001) software packages as well as evolutionary conservation were used to predict the pathogenic character of the sequence alteration.

A sequence variant was considered to be pathogenic if it represented a nonsense or frameshift mutation and co-segregated with the phenotype. A sequence variant was considered to be of likely pathogenicity if it affects an evolutionary conserved residue on amino acid and nucleotide level, if it was predicted by one or more programs mentioned above to be pathogenic, appeared only 1-3 times in 209 patients and did not show up in at least 188 control alleles. Variants assumed to be most likely non pathogenic did not affect conserved amino acids or nucleotides, represent silent mutations, did not co-segregate with the phenotype, were predicted by both programs to be benign, appeared in >3 patients or showed up in controls.

RESULTS AND DISCUSSION

Mutation screening of *C2orf71* by direct sequencing of all coding exons was performed on 209 index patients with a diagnosis of arRP. Of these, 2 patients were compound heterozygous for mutations, which are predicted to lead to premature stop codons (Table 1, first part, Figure 1). Patient CIC00261 from family 180 (II-1 in Figure 1A) was heterozygous for a deletion (c.946delA) (reviewed but not shown) resulting in a frameshift mutation p.Asn316MetfsX7. This would lead to a truncation of more than 80% of the C2orf71 protein. Co-segregation analysis revealed that this change was inherited from the unaffected mother (I-2, Figure 1A). The same deletion has been reported by Collin et al. as a homozygous mutation in a Dutch family (Collin et al. 2010). Our patient's family originated from the Southwest of France but the mother did not precisely know her ancestral history so a Dutch descent with a founder effect could not be excluded. In addition, CIC00261 was heterozygous for a novel nonsense mutation (c.3002G>A transition), which is predicted to lead to a stop codon (p.Trp1001X) (reviewed but not shown). Co-segregation analysis revealed that this change was inherited from the unaffected father (I-1). The unaffected brother (II-3) showed two normal alleles (Figure 1A). Patient CIC02622 from family 927 (II-3 in Figure 1B) was heterozygous for a novel nonsense mutation (c.2950C>T) leading to a stop codon (p.Arg984X) (reviewed but not shown), which was inherited from the unaffected father (I-1, Figure 1B). In addition, the patient was heterozygous for a novel duplication (c.2327dupC), which is predicted to lead to a frameshift and premature truncation of the protein (p.Leu777PhefsX34) (reviewed but not shown). This change was inherited from the unaffected mother (I-2, Figure 1B). Similar to the index patient, the affected brother (II-5, Figure 1B), was heterozygous for both mutations while the unaffected brother (II-1, Figure 1B) carried only the nonsense mutation.

Table 1: *C2orf71* variants which are pathogenic and likely pathogenic

Exon	Sequence variation	Protein effect	Frequency in patient chromosomes	Frequency in control chromosomes	Polyphen Prediction	SIFT Prediction	Conservation
1	c.946delA	p.Asn316Metfs*7	1/418	^a 0/242	n.a.	n.a.	n.a.
1	c.2327dupC	p.Leu777Phefs*34	1/418	0/188	n.a.	n.a.	n.a.
1	c.2950C>T	p.Arg984*	1/418	0/188	n.a.	n.a.	n.a.
1	c.3002G>A	p.Trp1001*	1/418	0/188	n.a.	n.a.	n.a.
1	c.1114G>A	p.Asp372Asn	1/418	0/188	benign	affects protein function	conserved
1	c.1835T>C	p.Leu612Pro	1/418	0/192	possibly damaging	tolerated	conserved
1	c.1844T>A	p.Val615Asp	2/418 ^b 1/572	0/192 ^b 0/190	possibly damaging	tolerated	conserved

^aperformed by (Collin et al. 2010)

^bperformed by (Sergouniotis et al. 2010)

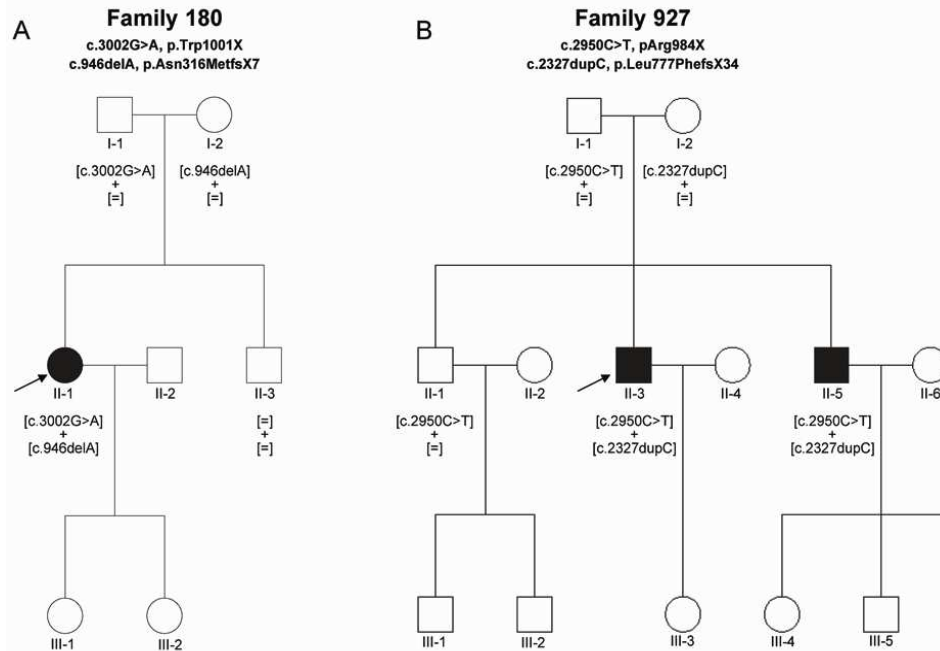


Figure 1: *C2orf71* mutations and co-segregation in 2 families with arRP. Filled symbols represent affected and unfilled unaffected subjects. Squares indicate males and circles females. Arrows reflect the index patients. The equal signs depict the normal allele.

Clinical data of index patients for the 2 families are summarized in table 2 and colour fundus and autofluorescence images are displayed in figure 2. Besides a variable age of onset and degree of visual impairment, both patients showed severe retinal dysfunction with marked macular atrophy. Similar clinical findings were reported by Collin and colleagues (Collin et al. 2010). *C2orf71* mutations are therefore associated with severe RP and early cone involvement, which highlights the importance of this gene for normal photoreceptor function.

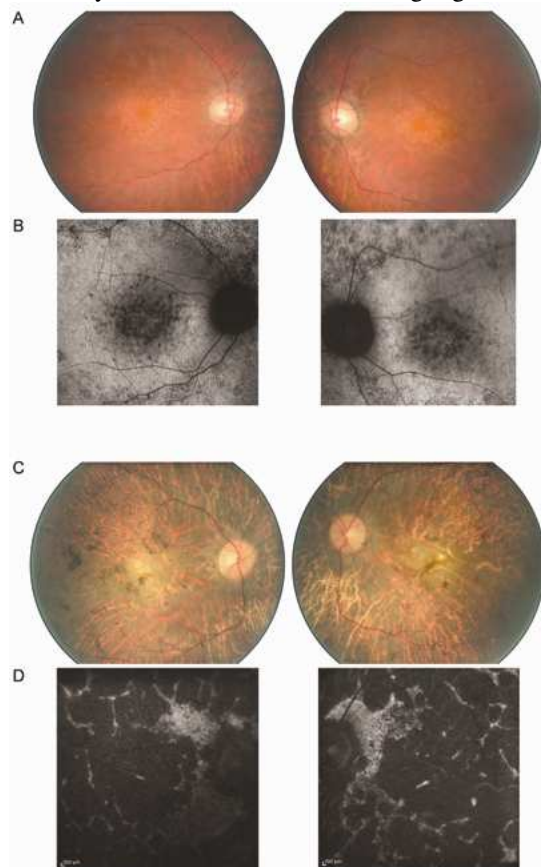


Figure 2: Colour fundus photographs (A-C) and autofluorescence imaging (B-D) of two index patients with *C2orf71* mutations leading to a premature stop codon. (A-B) CIC00261 showed little pigmentation in retinal periphery, some optic disc pallor and narrowed retinal vessels. In addition, there were macular atrophic changes which manifests by a patchy loss of autofluorescence; (C-D) CIC02622 showed marked atrophic changes in the posterior pole with large corresponding areas of loss of autofluorescence.

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Table 2: clinical data for the 2 patients compound heterozygous for mutations leading to premature stop codons

Patient	Age at time of diagnosis	Age at time of examination	Sex	Relevant medical and ophthalmic history	Family history	Symptoms	BCVA OD/OS Refraction	Colour vision	Binocular Goldman visual field, III4 isopter	Full field ERG	Multifocal ERG	Fundus and autofluorescence imaging (Figure 2)	Horizontal foveal thickness measured by SD-OCT*
CIC00261 Family 180 (II-1)	18	35	F	Refractive surgery for myopia at 25	None from Southwest of France	Night blindness at 18 ; visual field constriction and decreased vision at age 25	20/40 20/50 +0(-1)0° +0(-1.50)170°	tritanopia at the 28 Hue of Roth	40° both horizontally and vertically	UD	UD	Typical RP fundus changes with little pigment migration and some macular atrophic changes	OD 204µm OS 181µm
CIC02622 Family 927 (II-3)	Early childhood	44	M	none	One brother with RP from the West part of France	Life long night blindness and decreased vision	LP both eyes -3.50(-1)180° -3.25	NP	Inferior to 10°	UD	NP (unstable fixation)	Typical RP fundus changes with marked macular atrophic changes	OD 147µm OS 139µm

BCVA: best corrected visual acuity; OD: Oculis dextra (right eye); OS: Oculis Sinistra (left eye); UD: undetectable; NP: not performed; LP: light perception; RP: retinitis pigmentosa; SD-OCT: spectral-domain Optical Coherence Tomography; *normal value 227.64±11.74µm (Carpineto et al. 2010).

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C2orf71 screening of our cohort led to the identification of many other variants apart from these 4 premature stop mutations. These changes were classified as of likely pathogenicity (Table 1, second part), most likely non pathogenic (Table 3) and as known variants (Supplementary Table 2).

The group of variants with likely pathogenicity includes 3 missense mutations (Table 1 second part), which were evolutionary conserved (Supplementary Figure 1) and predicted by at least one program to be pathogenic. Unfortunately, no other affected family member of the respective index patients was available for genetic testing to prove the pathogenic character of those mutations. Patients carrying these mutations were only heterozygous for a single mutation. The p.Val615Asp variant was also detected in a patient in a different study investigating *C2orf71* variations in patients with Leber Congenital Amaurosis and arRP (Sergouniotis et al. 2010). As described here no second mutation was identified, but the mutation did not show up in control samples. To date, the 5'UTR of *C2orf71* is not described. In addition other isoforms of *C2orf71* may exist, which may harbor the second missing mutation. Since our screening strategy was based on direct sequencing of currently known coding exons and flanking regions, we may have missed a second change. This may also hold true for a number of isolated variants identified by Sergouniotis and co-workers (Sergouniotis et al. 2010). In addition, patients may have large heterozygous deletions, which can not be detected by our direct sequencing approach. Another possibility would be that these 3 variants are not pathogenic, that they represent rare variants or that the patients are only carriers. Our screening together with those by Sergouniotis and co-workers (Sergouniotis et al. 2010) showed indeed the highly polymorphic nature of *C2orf71*, which is concluded from the following results. Pursuing our stringent criteria, we identified 24 non-likely pathogenic variants, of which some were also identified by others (Collin et al. 2010; Sergouniotis et al. 2010). They comprise 15 missense changes, 8 silent mutations and 1 duplication and represent either frequent or very rare variants (Table 3). In addition, many patients were found to only carry one or more known SNPs (Supplementary Table 2). We conclude that all of them were not pathogenic since they appeared frequently in patients or controls in our study or in those performed by Sergouniotis and co-workers (Sergouniotis et al. 2010) or did not affect a conserved amino acid or nucleotide as it is the case for p.His122His Val247Ala and p.Pro1254Leu (data not shown).

All together, our study provides a large scale screening of *C2orf71* in a French cohort of arRP patients through direct sequencing. It identifies 2 families with 3 novel and 1 known mutations leading to premature stop codon and the absence of protein production as a putative disease mechanism. Furthermore, our study identifies 3 patients heterozygous for variants with uncertain pathogenic character. *C2orf71* would therefore account for at least 0.6-1.7% of arRP. This figure derives from PCR based exonic sequencing of 209 patients from a total of 345 ar and simplex RP patients. All 345 patients had been pre-screened by microarray or direct sequencing approaches for other RP causing mutations/genes. This pre-screening showed at least a single heterozygous putative mutation in other RP causing genes in 136 patients. Some of them carried two mutations and some of the mutations even showed familial segregation. Of note is that some of the heterozygous mutations amongst the 136 patients may have occurred by chance and therefore it is possible that some of these patients may actually carry a putative *C2orf71* mutation. If this were to be true then our estimated figure for *C2orf71* mutation (0.6-1.7%) is likely to go up since the figure is based on a total of 345 patients and not only on the 209 patients who have been sequenced in *C2orf71*.

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Table 3: *C2orf71* variants that are most likely non pathogenic

Exon	Sequence variation	Consequence	Frequency in patient chromosomes§	Frequency in control chromosomes	Polyphen Prediction	SIFT Prediction	Conservation	Co-segregation
1	c.679G>A	p.Glu227Lys	5 /418 b3/572	0/190 b0/382	benign	affects protein function	conserved	did not co-segregate in one family
1	c.773G>T	p.Arg258Ile	1/418	0/190	possibly damaging	tolerated	same nucleotide found in Stickleback	n.d.
1	c.935G>A	p.Ser312Asn	1 /418	0/188	benign	tolerated	not conserved on nucleotide level	n.d.
1	c.958C>T	p.Arg320Cys	1 /418	0/188	benign	tolerated	same amino acid found in rat and mouse	n.d.
1	c.1132G>A	p.Glu378Lys	1 /418	0/188	benign	tolerated	not conserved on nucleotide level	n.d.
1	c.1545A>G	p.Gln515Gln	1/418	n.d.	n.a.	n.a.	not conserved on nucleotide level	n.d.
1	c.1882G>A	p.Ala628Thr	2/418	0/192	benign	tolerated	not conserved on nucleotide level	n.d.
1	c.2063G>A	p.Cys688Tyr	4/418 b0/572	5/192 b3/190	probably damaging	tolerated	not conserved	n.d.
1	c.2502T>C	p.Pro834Pro	2/418 b1/572	n.d. b0/382	n.a.	n.a.	not conserved	n.d.
1	c.2600C>T	p.Pro867Leu	1/418 patient homozygous for this variant a1 b2/572	n.d. a1/180 b1/380	benign	tolerated	not conserved	n.d.
1	c.2860C>T	p.Pro954Ser	1/418	0/190	possibly damaging	tolerated	not conserved on nucleotide level	n.d.
1	c.2864G>A	p.Arg955Gln	3 /418 b0/572	2/190 b1/190	benign	tolerated	not conserved	n.d.

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1	1	c.2875G>A	p.Ala959Thr	2/418 1/572	0/190 ^b 2/190	benign	tolerated	not conserved on nucleotide level	n.d.
2	1	c.2889C>T	p.Ser963Ser	6/418 ^b 3/572	2/190 ^b 2/190	n.a.	n.a.	not conserved	n.d.
3	1	c.3058_3059delCA insAG	p.Gln1020Arg	6/418 ^b 3/572	3/190 ^b 2/372	benign	tolerated	conservation not known	n.d.
4	1	c.3478G>A	p.Ala1160Thr	1/418	0/190	benign	tolerated	conservation not known	n.d.
5	1	c.3530G>A	p.Arg1177Gln	2/418	0/190	benign	tolerated	conservation not known	did not co-segregate with the disease in one family
6	1	c.3291G>A	p.Gln1097Gln	6/418 ^b 3/572	5/190 ^b 1/372	n.a.	n.a.	not conserved	n.d.
7	1	c.3447G>A	p.Pro1149Pro	1/418 ^b 5/572	1/190 ^b 1/174	n.a.	n.a.	not conserved	n.d.
8	1	c.3522C>T	p.Asp1174Asp	1/418 ^b 2/572	1/190 ^b 1/174	n.a.	n.a.	not conserved	n.d.
9	2	c.3739G>A	p.Gly1247Ser	4/418 ^b 4/572	2/192 ^b 3/300	benign	tolerated	conservation not known	n.d.
10	2	c.3672_3674dup ^o	p.Ser1225dup	105 patients heterozygous, 23 patients homozygous/418	7/192	n.a.	n.a.	conservation not known	n.d.
11	2	c.3840G>A	p.Ala1280Ala	1/418 ^b 2/572	0/192 ^b 0/300	n.a.	n.a.	not conserved	n.d.
12	2	c.3789G>A	p.Leu1263Leu	1/418 ^b 4/572	0/192 ^b 1/300	n.a.	n.a.	conserved on nucleotide level	n.d.

§ except where it is noted, the variants occurred heterozygously

^aperformed by (Collin et al. 2010)

^bperformed by (Sergouniotis et al. 2010)

^o located in a highly polymorphic region with 2 known SNPs in the same region rs72183347 and rs72122505

- n.d. not done
- n.a. not applicable

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Interestingly, another prevalence study from a cohort with 286 affected individuals with Leber Congenital Amaurosis and arRP identified a large number of variants in *C2orf71*, however for none of the variants pathogenicity could be proven (Sergouniotis et al. 2010).

Although our study documents that *C2orf71* is not a major gene for arRP, however at least for the French arRP population it is still an important gene to screen and its prevalence is similar to most of the other genes reported in arRP (Hartong et al. 2006).

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SUPPORTING FILES:

Supplementary Figure 1:

	372	612	615
	N	P	D
Human	KQTSWDL-APEP	PTFQELRRVQR	QELRRVQRDLS
Rhesus	KQTSWDL-APEP	PTLQELQRVQR	QELQRVQRDLS
Rat	KQASWDF-AAEL	PTLQELRRVQT	QELRRVQTDLS
Mouse	KQASWDF-AAEL	PTLQELRRVQT	QELRRVQTDLS
Opossum	KQASLDS-LDEP	-----	-----
Platypus	TQASCDHPVQQA	-----	-----
Chicken	AQASF-----	-----	-----

Evolutionary conservation of altered amino acid residues (red) in other orthologs. Multiple amino acid alignments show evolutionary conservation of mutated residues (green).

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Supplementary Table 1: primer sequences used for *C2ORF71* PCR amplification and sequencing

Primer	Sequence (5'-3')
C2ORF71 1A F	CAACAGGACGACAGGTACCA
C2ORF71 1A R	GAAGCAGGATCTCCCATGAG
C2ORF71 1B F	CAATTCGGCCAGGATGTCAG
C2ORF71 1B R	GTGTAGATAGGTGTAAGCCTG
C2ORF71 1C F	GTCACACGTCAAGCACACAG
C2ORF71 1C R	GCTTATTTTCCAAGTGGGTTGC
C2ORF71 1D F	CAGGTGCTCAATGGCACAGT
C2ORF71 1D R	GAGATGAAGCGTCCATCGGC
C2ORF71 1E F	GTACAAGCCCAGAAAATATCAC
C2ORF71 1E R	GTGACTCTGGAGACACGACT
C2ORF71 1F F	CGAAAGGATCAAGTTTGTCCC
C2ORF71 1F R	CTCTCCGTGGGACTGAAAGT
C2ORF71 1G F	CATCCTGAGGACGAACAAG
C2ORF71 1G R	CGAATGATTTGTCCATCAGAAC
C2ORF71 1H F	CTGCCTAAAGCAGAAGCAGC
C2ORF71 1H R	GAGGTCCTGTTTTGTCCAGAT
C2ORF71 1I F	GCCTGACCAAGCCTCACAG
C2ORF71 1I R	GATGGGGAGGGAATCGAGAA
C2ORF71 1J F	CACCACAAAGCGGCGAACTT
C2ORF71 1J R	GAAGATCTTTTGGCCCATTTCG
C2ORF71 2 F	GTTGCTTTGTTTCAGAGCAGCA
C2ORF71 2 R	CTCAGTAGGAGTTTGGTTTGC

MUTATION IN BRIEF

HUMAN MUTATION

Supplementary Table 2: *C2orf71* known non-disease causing variants in our cohort of 209 arRP patients with their respective frequency

Exon	Sequence variation	Consequence	Frequency in patient chromosomes	Frequency in control chromosomes	SNP ID
1	c.37A>T	p.Ser13Cys	15 /418 ^b 1/572	n.d. ^b 0/364	rs10084168
1	c.60G>A	p.Gln20Gln	120/418 ^b 101/572	n.d. ^b 82/364	rs35929540
1	c.258G>A	p.Arg86Arg	120/418 ^b 111/572	n.d. ^b 77/364	rs62132765
1	c.366C>T	p.His122His	1/418	n.d.	rs17007546
1	c.740T>T	p.Val247Ala	1/418	0/190	rs77828062
1	c.755C>A	p.Ala252Asp	10/418	1/190	SNP2-29149877
1	c.1262A>G	p.Lys421Arg	12/418 ^b 0/572	n.d. ^b 3/190	rs17007544
1	c.1452C>T	p.Ser484Ser	151/418 ^b 153/572	n.d. ^b 81/190	rs13385188
1	c.1739C>T	p.Thr580Met	33/418 ^b 0/572	n.d. ^b 5/190	rs10166913

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1	c.1942G>C	p.Ala648Pro	4/418 ^b 1/572	n.d. ^b 1/190	rs75276619
1	c.2112T>C	p.Asn704Asn	153/418 ^b 168/572	n.d. ^b 123/380	rs10200693
1	c.2374C>G	p.Leu792Val	120/418 ^b 103/572	n.d. ^b 66/380	rs17744093
1	c.2499G>A	p.Pro833Pro	121/418 ^b 106/572	n.d. ^b 70/380	rs34253433
1	c.3264_3266del	p.Pro1089del	11/418	n.d.	rs75460934
2	c.3761C>T	p.Pro1254Leu	1/418	0/190	rs1975713

^bperformed by (Sergouniotis et al. 2010)