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Genetic Analysis of *FAM46A* in Spanish Families with Autosomal Recessive Retinitis Pigmentosa: Characterisation of Novel VNTRs

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Summary

Retinitis pigmentosa (RP) is a group of retinal dystrophies characterised primarily by rod photoreceptor cell degeneration. Exhibiting great clinical and genetic heterogeneity, RP be inherited as an autosomal dominant (ad) and recessive (ar), X-linked (xl) and digenic disorder. *RP25*, a locus for arRP, was mapped to chromosome 6p12.1-q14.1 where several retinal dystrophy loci are located. A gene expressed in the *retina, FAM46A*, mapped within the *RP25* locus, and computational data revealed its involvement in retinal signalling pathways. Therefore, we chose to perform molecular evaluation of this gene as a good candidate in arRP families linked to the *RP25* interval. A comprehensive bioinformatic and retinal tissue expression characterisation of *FAM46A* was performed, together with mutation screening of seven RP25 families.

Herein we present 4 novel sequence variants, of which one is a novel deletion within a low complexity region close to the initiation codon of *FAM46A*. Furthermore, we have characterised for the first time a coding tandem variation in the Caucasian population.

This study reports on bioinformatic and moleculardata for the *FAM46A* gene that may give a wider insight into the putative function of this gene and its pathologic relevance to RP25 and other retinal diseases mapping within the 6q chromosomal interval.

Keywords: retinitis pigmentosa, *RP25* locus, retinal disease gene, chromosome 6

Introduction

Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies, characterised by photoreceptor cell degeneration leading to night blindness and visual field loss. Clinical manifestations include pigment deposition in the retina and attenuation of retinal blood vessels, with later depigmentation or atrophy of the retinal pigment epithelium. Electroretinogram (ERG) changes are present, with abnormalities of both rod and cone functions, with the scotopic alteration the first to be reported followed by undetectable photopic responses in advanced cases of RP (Jiménez et al. 1989). RP is the most frequent form of retinal dystrophy worldwide, affecting approximately 1 in 4000 people (Inglehearn, 1998).

Apart from its clinical variability, and probably for this reason, RP shows notable allelic and non-allelic heterogeneity, and can be inherited as an autosomal dominant (ad), autosomal recessive (ar), X-linked (Xl) and digenic condition.

Autosomal recessive (arRP) is the commonest form of RP worldwide, and accounts for approximately 39% of all cases in Spain (Ayuso, 1995). It can be caused by mutations

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in at least 20 genes (http://www.sph.uth.tm.edu/Retnet/). *RP25*, one of the loci for which the gene is yet to be revealed, was originally mapped in 4 Spanish families with arRP in a region of approximately 16.1 cM between markers *D6S257* and *D6S1644* (Ruiz et al. 1998). Recently, evidence of linkage to the same region has been reported in three additional Spanish arRP families, as well as in other ethnic groups (Barragan et al. 2005a; Abd El-Aziz et al. 2006).

According to the Retinal Information Network (http://www.sph.uth.tm.edu/Retnet/), both chromosomes 1 and 6 harbour the majority of non-X linked retinal loci (Castori et al. 2005). Interestingly, 6 loci have been reported as responsible for various types of dominant and recessive retinal dystrophies within the 6q interval and colocalize with the *RP25* locus, including a form of Leber congenital amaurosis (*LCA5*) (Dharmaraj et al. 2000), dominant cone-rod dystrophy (*CORD7*) (Kelsell et al. 1998), North Carolina type dominant macular dystrophy (*MCDR1*) (Small et al. 1992), and autosomal dominant Stargardt-like disease (*STGD3*) (Stone et al. 1994), among others.

Extensive genetic analysis of putative genes within the *RP25* locus interval has led to the exclusion of a number of them as disease-causing (Marcos et al. 2000, 2002, 2003; Li et al. 2001; Abd El-Aziz et al. 2005, 2006; Barragan et al. 2005a; Barragan et al. 2005b).

FAM46A, originally named *C6orf37*, a retinally-expressed gene, was reported as a good candidate for human retinal diseases since it demonstrated preferential expression within the neural retina. The gene is localised within the critical interval for the *RP25* locus where the loci for 3 additional retinal degenerations, autosomal dominant atrophic macular degeneration (adMD1), *STGD3* and *CORD7*, have been identified. Even though this gene has been excluded as causative for the above three loci (Lagali et al. 2002), we felt it was mandatory to screen it in RP25 families.

In addition, structural features, interaction with proteins involved in cellular mechanisms associated with neurodegenerative diseases and retinal development and homeostasis, and the extent of sequence conservation, suggested a putative role for FAM46A in cell signalling pathways related to retinal degeneration (Lim et al. 2006; Colland et al. 2004; Beier et al. 2006; Sehgal et al. 2006; Yu et al. 2004). Furthermore, the finding that *FAM46A* is up-regulated by angiotensin II, which has recently been isolated in retinal neurons where it may act as a neuromodulator, supports our hypothesis of *FAM46A* involvement in the pathogenesis of RP (Romero et al. 2004; Savaskan et al. 2004; Guenther et al. 1996).

Herein, we describe a further comprehensive bioinformatic and retinal tissue expression characterisation of the *FAM46A* gene, together with its molecular evaluation as a pathogenic gene in 7 arRP families linked to the *RP25* locus.

Materials and Methods

Subjects and DNA

This study involved arRP affected patients belonging to 7 unrelated families linked to *RP25* locus, which was previously mapped to chromosome 6p12.1-q15 by our group (Ruiz et al. 1998). As described previously the participating families conform to the phenotypic and inheritance patterns of arRP.

The study protocol was in accordance with the tenets of the Declaration of Helsinki (June, 1964). A group of control individuals was also recruited which comprised unselected, unrelated race-, age-, and gender-matched individuals from Spain. Informed consent was obtained from all members participating for a clinical and molecular genetic study. Ten ml peripheral blood was taken from each individual for genomic DNA purification from leukocytes using standard protocols.

Bioinformatic Analysis

The genome sequence of the *FAM46A* gene, as well as transcript and protein structure and evolutionary conservation data, was accessed through the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), Ensembl and Vega databases (http://www.ensembl.org; http://vega.sanger.ac.uk) and the UCSC human genome browser (http://genome.ucsc.edu/). Information on expression patterns was obtained from UniGene's EST ProfileViewer and the NCBI Gene Expression Omnibus database at NCBI, along with the EBI ArrayExpress Warehouse 6.11 tool at the European Bioinformatics Institute (EBI, http://www.ebi.ac.uk). Specific retinal transcripts were investigated in Retina Central (http://www.retinacentral.org), a platform for gene-related information on the adult mammalian retina. The Annotated Human Gene Database was queried for data about the cellular location, associated metabolic pathways and characterization of the protein motifs of FAM46A.

Searches for molecular interactions were carried out using the PIMRider visualization platform, through which a graphical and dynamic view of the protein interaction networks can be displayed for a given protein (https://pim.hybrigenics.com/pimriderext/tgf-beta/fullbaitlist.html). Some of the FAM46A partners were cross-checked in interaction databases such as IntAct at EBI.

Expression Study

The *FAM46A* gene was PCR-amplified for cDNA from the human retina, in order to assess its retinal expression, as well as to determine which alternatively spliced version of the transcript

was present in this tissue, and to confirm that this coincided with the bioinformatic prediction. Subsequently, two-sense sequencing of the amplified fragments was performed in order to detect the coding region and the initiation codon (ATG) present in the transcripts found in the retina.

Mutation Screening

The molecular analysis of FAM46A was performed in the index patients of the *RP25* linked families (RP5, RP73, RP167, RP214, RP235, RP260 and RP299). Nine pairs of primers were designed to ensure total PCR coverage of the entire coding region, the intronic flanking sequences, the regulatory factor binding sites and the 5′ and 3′ untranslated regions (UTRs) of the major transcript, as well as the additional exons contributed by other published alternatively spliced isoforms (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi).
In addition, a tenth pair of primers was designed to amplify a region that comprises two of the identified genetic variants in a single fragment. Once amplification was accomplished the resulting PCR products were subsequently purified using an enzymatic procedure according to the manufacturer's recommendations (ExoSAP-IT®, USB Corporation) and screened for mutations by direct sequencing analysis (automated sequencer ABI 3130xl, Applied Biosystems).

Finally, the sequence data was analysed using SeqMan™ 4.03 software (DNASTAR Inc). Consecutively, the newly identified intronic sequence variations were submitted to Splice Site and Transcription Factor Binding sequence prediction interfaces (http://www.fruitfly.org/seq_tools/splice.html, http://www.cbil.upenn.edu/cgi-bin/tess)

Genotyping of the Identified Changes

Those variants identified in the low complexity regions were genotyped by fragment analysis, using a pair of primers designed to amplify specifically the gene interval that harboured both variations, with the primers labelled at the 5′ end with 6-FAM fluorocrome. Later sequencing served as a reproducibility control for the genotyping data, as well as for further genotyping of all unpublished variants found in the initial mutation screening.

PCR and electrophoresis conditions and primer sequences employed are available on request.

Statistical Analysis

Allelic and genotype frequencies were calculated for each of the identified variants, in both the affected and control populations. Mendelian transmission of the changes was confirmed by allelic co-segregation. Additionally, statistical characterization of the identified variable number tandem repeat (VNTR) consisted of heterozygosity (HET) and polymorphism information content (PIC) calculations for our samples.

Results

Bioinformatic Analysis

Encompassing around 7 Kb of the genomic sequence in 6q14, flanked by the microsatellite markers *D6S1495* and *D6S1882*, *FAM46A* is organised into 3 exons separated by intervening intronic regions spanning 251 and 1117 nucleotides respectively. Bioinformatic analysis of *FAM46A* using a variety of sequence and genome annotation databases led to the identification of 5 different isoforms, varying in the length of the first exon and the position of the ATG, as well as in the presence of alternative exons, therefore producing proteins ranging from 86 to 523 amino acid residues. Among these full-length transcripts, and according to the EMBL/GenBank/DDBJ databases and the literature, the eye-expressed isoform encodes a 442 amino acid (aa) protein with a molecular weight of ∼50 Kd and a theoretical isoelectric point of 5.03. Such an isoform encoding this variant was confirmed by PCR amplification, together with direct sequence analysis of retinal cDNA. The subcellular location of the protein is reported in the databases as cytosolic, making it possible to predict that FAM46A is a soluble protein. FAM46A has been identified as a bait in a Yeast Two-Hybrid Screening performed to generate a protein-protein interaction map of the Smad human signalling pathway (Colland et al. 2004). These data, reported through the PIMRider visualization platform, indicated that FAM46A is a Smad signalling pathway related protein, also showing that SARA, BAT3 and DNCH1, among other proteins, act as its interaction partners. Moreover, other interaction databases show that FAM46A interacts with ATXN1 (Spinocerebellar ataxia type 1 protein). According to its expression pattern, it is interesting to note that the eye and retinoblastoma are shown as sources for isolation of *FAM46A* cDNA; besides, this gene is found in the retinome database (http://www.retinacentral.org/) and, albeit that it is widely expressed, preferential expression has been observed in the retina compared to other ocular tissues. Additional "*in silico*" findings, such as evolutionary conservation patterns, phosphorylation sites, protein structure, and the presence of AU-rich motifs and repetitive sequences conformed to the previously published data (Lagali et al. 2002; Cui et al. 2006). One of the FAM46A protein domains, DUF1693, is also encoded by other human genes that have been organised into a gene family, FAM46 ("family with sequence similarity 46"), which gave this gene its current name. Besides, this domain has been found to be conserved among diverse orthologs.

FAM46A featurs 2 low complexity regions, confirmed by sequencing analysis. The first one, located within intron 1, contains a 19 nucleotide repeat motif, whereas the second is composed of 15 tandem nucleotide units

Table 1 Sequence variations detected in the molecular analysis of the FAM46A gene

Sequence variation	AA change	SNP database ID	Allele frequency		Genotype frequency			
			Patients	Controls	Patients		Controls	
					−/+	+/+	−/+	+/+
c.-16G>T	-	Not published	0.17	0.05	0.18	0.08	0.09	0.00
c.-78C>T	-	Not published	0.06	0.02	0.12	0.00	0.03	0.00
c. 74G>A	p. G25D	Not published	0.00	0.01	0.00	0.00	0.02	0.00
c. 654T>C	p. I113I	rs3186631	0.03	0.10	0.06	0.00	0.16	0.01
c. 242A>T[#]	p. H81L	rs6935556	0.04	-	0.02	0.00	-	-
c. *55G>A	-	rs11755003	0.11	-	0.06	0.00	-	-
c.-88_-67delTGCTCTCCGCCGCCGCGCG	-	-	0.03	0.09	0.06	0.00	0.15	0.01
c. 70(GGCGGCGACTTCGGC)2–7	p. 24(GGDFG)2–7	-	§	§	§	§	§	§

+Represents the polymorphic allele.
#Variations corresponding to the alternative transcript OTTHUMT00000041329. The nomenclature employed for the variations identified in the present work conform to the recommendations of http://www.hgvs.org/mutnomen/.
§Frequencies of the coding tandem variation alleles and genotype frequencies of the 19 bp deletion combined with the tandem variation are described in detail in Table 2.

(GGCGGCGACTTCGGC)n, and conforms to a glycine-rich region (PS50315 domain). Sequence analysis of retinal cDNA upheld the fact that the latter region is actually part of the transcript that is expressed in the human retina. Although they are generally not very well understood, these regions have been shown to be functionally important in some proteins, and thus have been evolutionary conserved, specifically in this family branch.

Molecular Analysis

Sequence analysis was conducted to test whether *FAM46A* was implicated in the pathogenesis of RP in our families linked to the *RP25* locus. As a result of the mutation screening 8 genetic variants were found, of which 4 were novel, and the remainder had been reported previously (Table 1). The polymorphic variants were assigned a nucleotide position, starting from the first translation base of *FAM46A* according to the NCBI Reference Sequences (RefSeq) entry NM_017633.2. For the sequence variants identified in one of the additional exons, corresponding to *FAM46A* alternative isoform 2, the VEGA transcript OTTHUMT00000041329 was employed as reference. It is important to note that all the changes detected co-segregated according to the original genetic data of the studied families (Ruiz et al. 1998; Barragan et al. 2005a). The group of novel variantsfound in this study comprised 3 single nucleotide polymorphisms (SNPs) and a 19 bp deletion (Table 1). Of the novel SNPs a G>T transversion was identified in the non-coding region of exon 2, 16 nucleotides upstream of the ATG, in the RP5, RP73 and RP167 families. This change was also present in the control population with a heterozygote frequency of 0.08, whereas no homozygotes were found among 100 control individuals (Tables 1 and 2). Transcription Element Search System results indicated that this nucleotide substitution is responsible for the loss of a transcription factor binding site.

The second novel variant identified in this work was an intronic transition (c.-78C > T) located 78 basepairs 5′ of exon 3. It appeared only in the RP73 and RP214 parents and unaffected members, respectively, among all the RP25 families, as well as in 3% of control individuals.

A third unpublished sequence variant was found when testing the control population for the former changes; this rare variant (1% frequency) represented a new coding SNP, which consisted of a non-conservative amino acid substitution of the neutral glycine to the acidic aspartic acid at codon 25 of the FAM46A protein.

Finally, the novel deletion of 19 nucleotides was positioned 67 basepairs before the ATG codon, 28 nucleotides from the beginning of exon 2. This deletion significantly affected one of the low complexity regions previously mentioned, and was localised only 7 nucleotides away from the splicing consensus acceptor region ($(C/T)_n$ N C/T AG G/A) and associated with the loss of 5 predicted transcription factor binding sites. It co-segregated with the disease assuming compound heterozygosity in one of the families (RP73); however, it was also detected in control individuals with an allele frequency of 0.09.

Additionally, a minisatellite tandem repeat consisting of 15 nucleotides, which encode a 5 amino acid repeat region (GGDFG), was identified in the exon 2 segment corresponding to the second low complexity region characteristic of this gene. This tandem repeat variation exhibited 6

A
c. 70(GGCGGCGACTTCGGC)n

	Allele frequency			Genotype frequency	
n	Patients	Controls	n/n	Patients	Controls
2	0,04	0,00	**2/3**	0,06	0,00
			2/5	0,02	0,00
3	0,18	0,28	**3/3**	0,02	0,07
			3/4	0,08	0,06
			3/5	0,14	0,21
			3/6	0,04	0,07
4	0.18	0,18	**4/4**	0,02	0,08
			4/5	0,18	0,09
			4/6	0,02	0,01
5	0,48	0,46	**5/5**	0,22	0,19
			5/6	0,08	0,01
6	0,11	0,07	**6/7**	0,00	0,01
7	0,00	0,01	**7/5**	0,00	0,01

B
c.-88_-67delTGCTCTCCGCCGCCGCGCG and c. 70(GGCGGCGACTTCGGC)n

		Genotype frequency	
	n/n	Patients	Controls
+/−	**-**	**0.00**	**0.00**
+/+	**-**	**0.00**	**0.00**
+/−	**3/4**	0,00	0,01
+/−	**3/5**	0,00	0,04
+/+	**3/5**	0,00	0,01
+/−	**4/5**	0,04	0,02
+/−	**5/5**	0,02	0,06
+/−	**5/6**	0,00	0,02

Table 2 *Allelic and genotype frequencies* (Allelic and genotype frequencies of the tandem variation in patients and control population (A) and comparative genotype frequencies of the 19 bp deletion alone or appearing along with the tandem variation (B))

clearly distinguishable alleles, representing 2 to 7 unit repeats, that were visualized by fluorescent PCR-amplified fragment analyses. Co-segregation analysis of this tandem repeat in all the members of the *RP25*-linked families was performed in order to test it as a polymorphic marker for linkage studies (Figure 1). We characterized this novel marker in the Spanish population by determination of the allele and genotype frequencies in patients and controls and by measures of variation. The heterozygosity (HET) observed was 0.6726 and the polymorphism information content (PIC) was 0.65.

While the allele containing 4 repeats represented the annotated RefSeq reference sequence, the commonest allele in our study population turned out to be the 5 repeat units one, which also represented the most frequent homozygous genotype, followed by the sequence with 3 consensus repeats. It is noteworthy that the previously mentioned 19 nucleotide deletion was more frequent when associated with this tandem variation (Table 2, B).

Interestingly, one of the identified alleles containing the fewest repeat units was present exclusively in family RP299, and was not observed in any of the 100 screened controls.

Regarding the distribution of genotype frequencies, of interest was the fact that the genotypes composed of any combination with the fewest repeats (2 repeats) were only present in the RP25 families. Indeed, the combination composed of 2 and 3 repeat units was only found in the affected members of the RP299 family, whereas the higher repeat alleles were restricted to the non-affected family members (Figure 1).

Discussion

In the present study we have reported on an extensive bioinformatic analysis of *FAM46A* and its molecular screening as the gene responsible for the RP phenotype in arRP families linked to the *RP25* locus.

FAM46A spans around 7 Kb within the chromosome 6q region where the arRP locus *RP25* has been mapped (6p12.1-q15), together with several other retinal dystrophy loci (Dharmaraj et al. 2000; Small et al. 1992; Kelsell et al.

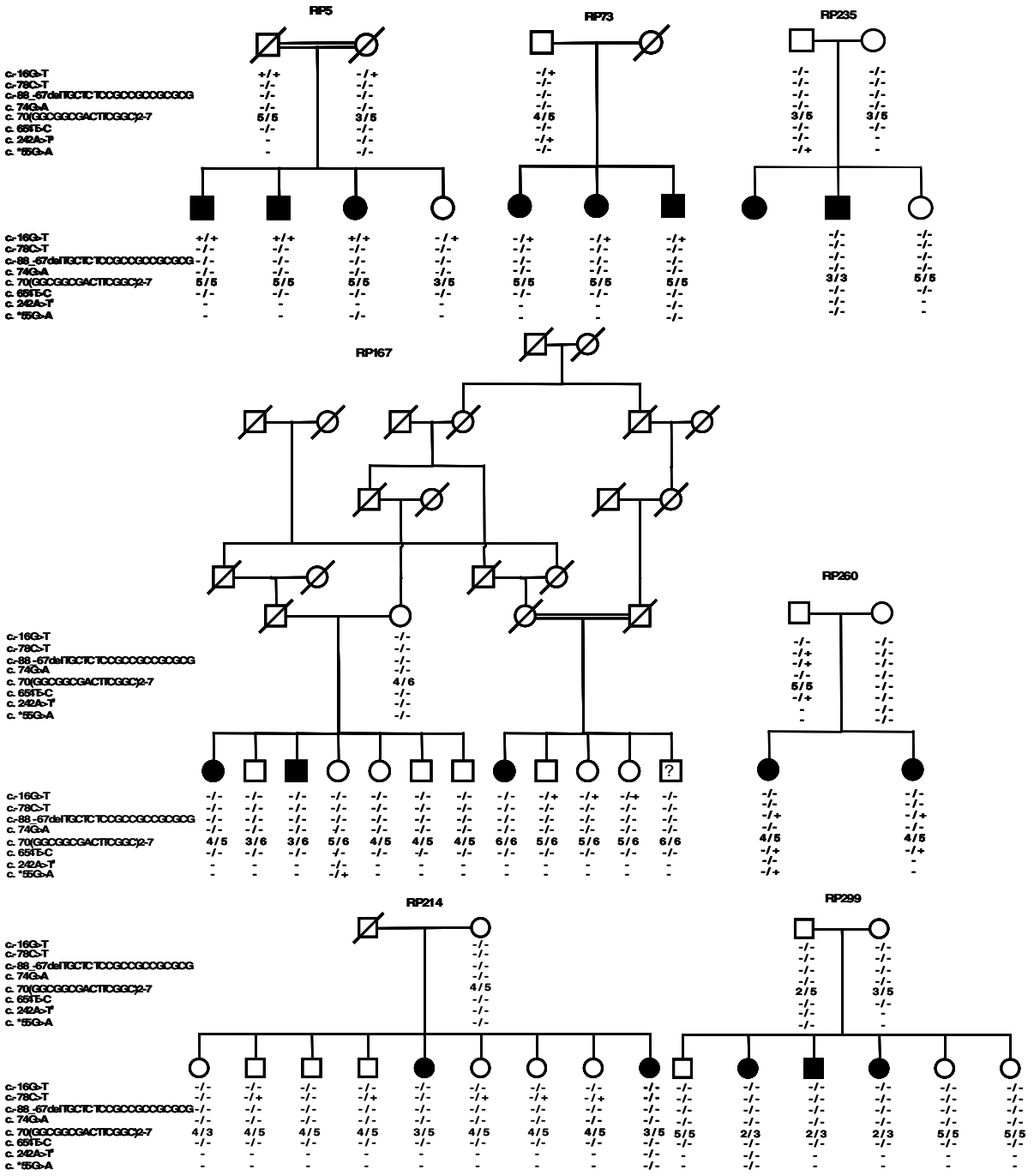

Figure 1 RP25 family trees showing the segregation of all sequence variants identified in the molecular analysis of *FAM46A*. *Filled symbols*: patients with arRP; *symbols with diagonal slash*: deceased members; *question mark*: probably affected.

1995). The fact that clusters of genes mapping in close proximity to each other on the same chromosome may cause similar phenotypes has already been noted (George, 2004). Furthermore, the frequent clinical heterogeneity reported in retinal degenerations (Cremers et al. 1998; Wells et al. 1993) makes it conceivable that different mutations in the same gene could predispose to variable phenotypes. However, previously identified genes responsible for other retinal degeneration loci mapping in the *RP25* region have already been excluded as being causative in *RP25*-linked families (Barragan et al. 2005a; Li et al. 2001; Abd El-Aziz et al. 2005).

Overall, the available information on the *FAM46A* gene suggests a relevant functional role for this gene in the retina.

First of all, the preferential expression of this gene within the neural retina over other eye tissues denotes a localised action; indeed, we have amplified *FAM46A* from retinal cDNA, thus confirming its previously and extensively reported retinal expression (Lagali et al. 2002; Schulz et al. 2004).

Secondly, subcellular location data and structural features such as the presence of conserved potential phosphorylation sites and multiple ATTTA sequence motifs in the 3′-UTR indicate that *FAM46A* may be involved in signal transduction processes (Lagali et al. 2002). Furthermore, the finding that FAM46A is a partner in the protein-protein interaction map of the Smad signalling pathway, specifically of SARA (Smad anchor for receptor activation), a key regulator of Smad activation, reinforces our hypothesis that this gene is implicated in cellular signalling, and provides additional evidence for its putative retinal function (Colland et al. 2004). Smads are intracellular effectors of signalling pathways of TGF-β (Derynck & Zhang, 2003), a superfamily of proteins also known to be expressed in the retina where they have a much reported role (Beier et al. 2006; Mathura et al. 2000; Honjo et al. 2007). Special relevance is attached to the finding that BMP7 increases the number of outer segment processes in chick cultured photoreceptors (Sehgal et al. 2006); in addition, other Smad-associated factors such as interleukin 2 and STAT-1 (signal transducer and activator of transcription-1) have been related to photoreceptor survival and retinal degeneration (Honjo et al. 2007). Finally, Smad proteins are also regulated by other kinase pathways (Derynck & Zhang, 2003), which have been proposed to cooperate with the regulation of cytoskeletal and Ca^{2+}-dependent proteins (Yu et al. 2004).

An additional association of the family of genes with neurodegenerative diseases has recently been published (Lim et al. 2006). Ataxia-causing proteins, reported to be part of the retinome (Schulz et al. 2004), are also classified as interaction partners of both FAM46A and SARA; interestingly, some ataxias are always associated with retinal degeneration (Helmlinger et al. 2006). Finally, *FAM46A* has been revealed as one of the genes, among relevant genes expressed the retina such as M-Ras or RhoB GTPase, that are immediate-early regulated genes when treated with angiotensin II, which has interestingly been localized in the inner segment of photoreceptor cells (Romero et al. 2004; Savaskan et al. *2004)*. Electrophysiological studies have suggested that angiotensin can act as a neuromodulator in the visual system through its effects on neuronal Ca^{2+} channels (Guenther et al. 1996).

The identification of a unique genotype (2/3) for the coding minisatellite variation, detected only in the affected individuals of the non-consanguineous family RP299, may be suggestive of a putative relationship with RP in this family. Assuming the clustering of retinal dystrophy loci, the great genetic heterogeneity of RP, and the high prevalence of the *RP25* gene in arRP, it is reasonable to conceive that there may be distinct genes responsible for RP in different arRP families mapping to *RP25*. There is evidence that transcribed VNTRs may affect both the mRNA and gene product stability/activity, as well as the pathogenic significance of the length of glycine repeats (Armour, 2006; Nakamura et al. 1998; Brito et al. 2005). The identified glycine-rich repetitive sequence would have a tendency to form turn and coil configurations, and an extremely low number of repeats may influence the protein structure to some extent. Therefore, some alleles may influence the retinal degeneration in family RP299. Alternatively, we could hypothesise that the presence of the lower repeat alleles only in RP-affected individuals may be reflecting linkage disequilibrium between such an allele and some other genetic change that influences the biological function of the gene (Verpy et al. 2000). Likewise, we cannot rule out the possibility that a second mutation lies in unscreened regions of the gene, such as the full intronic intervals and the UTR sequences (Deery et al. 2002).

The genotypes of this variant present in family RP167 led us to exclude this chromosomal region as a candidate for causing the disease in its non-consanguineous branch. Nevertheless, as it is conceivable that there may be distinct disease genes in different *RP25*-linked families, this region still constitutes a putative cause of *RP25* in the consanguineous branch, as well as in the rest of the linked families.

Furthermore, the detection of this tandem variation in the Caucasian population, and the identification of novel alleles and genotypes that are not present in the Chinese population, strengthen the evidence that this repetitive coding sequence is polymorphic and represent an additional insight into the population genetic diversity (Cui et al. 2006). Additionally, the heterozygosity and PIC information of this tandem repeat make it a new marker for linkage studies of *RP25*, as well as any other disease mapping to this interval.

Another relevant finding of this work has been the identification of a novel 19 bp deletion variant in the other low complexity region of *FAM46A*. Discarded as a causative mutation due to the presence of the homozygous deletion in the control population, the identification of this polymorphic sequence close to the initiation codon remains relevant, since extensive information has been published on the transcriptional regulation activity of untranscribed repetitive sequences (Soeby et al. 2005).

Our bioinformatic and expression characterisation, and mutation screening of *FAM46A* in RP-affected

individuals, have led us to conclude that this gene is likely to have a functional role in signalling pathways of the retina, and that affected individuals of the RP299 family present with a variant genotype that could be implicated in the activity of the FAM46A protein. Nevertheless, additional work on this and nearby genes is necessary to elucidate its involvement in the etiopathogenesis of RP.

We have also provided evidence for the existence of 2 repeat variants in the sequence intervals flanking the initiation codon that could function in the regulation of the gene at the transcriptional or translational levels. Incorporation of these findings will advance our understanding of the functionality and pathologic implication of *FAM46A* in RP25 and other retinal dystrophies mapping to the 6q interval.

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References

Abd El-Aziz, M. M., El-Ashry, M. F., Barragan, I., Marcos, I., Borrego, S., Antinolo, G. & Bhattacharya, S. S. (2005) Molecular genetic analysis of two functional candidate genes in the autosomal recessive retinitis pigmentosa, RP25, locus. *Curr Eye Res* **30**, 1081–1087.

Abd El-Aziz, M. M., El-Ashry, M. F., Chan, W. M., Chong, K. L., Barragan, I., Antinolo, G., Pang, C. P. & Bhattacharya, S. S. (2006) A Novel Genetic Study of Chinese Families with Autosomal Recessive Retinitis Pigmentosa. *Ann Hum Genet* **70**, 1–14.

Abd El-Aziz, M. M., Patel, R. J., El-Ashry, M. F., Barragan, I., Marcos, I., Borrego, S., Antinolo, G. & Bhattacharya, S. S. (2006) Exclusion of four candidate genes, KHDRBS2, PTP4A1, KIAA1411 and OGFRL1, as causative of autosomal recessive retinitis pigmentosa. *Ophthalmic Res* **38**, 19–23.

Armour, J. A. (2006) Tandemly repeated DNA: why should anyone care? *Mutat Res* **598**, 6–14.

Ayuso, C., Garcia-Sandoval, B., Najera, C., Valverde, D., Carballo, M. & Antinolo, G. (1995) Retinitis pigmentosa in Spain. The Spanish multicentric and multidisciplinary group for research into retinitis Pigmentosa. *Clin Genet* **48**, 120–122.

Barragan, I., Marcos, I., Borrego, S. & Antiñolo, G. (2005) Molecular analysis of RIMI in autosomal recessive retinitis pigmentosa. *Ophthalmic Res* **37**, 89–93.

Barragan, I., Marcos, I., Borrego, S. & Antinolo, G. (2005) Mutation screening of three candidate genes, ELOVL5, SMAP1 and GLULD1 in autosomal recessive retinitis pigmentosa. *Int J Mol Med* **16**, 1163–1167.

Beier, M., Franke, A., Paunel-Gorgulu, A. N., Scheerer, N. & Dunker, N. (2006) Transforming growth factor beta mediates apoptosis in the ganglion cell layer during all programmed cell death periods of the developing murine retina. *Neurosci Res* **56**, 193–203.

Brito, M., Malta-Vacas, J., Carmona, B., Aires, C., Costa, P., Martins, A. P., Ramos, S., Conde, A. R. & Monteiro, C. (2005) Polyglycine expansions in eRF3/GSPT1 are associated with gastric cancer susceptibility. *Carcinogenesis* **26**, 2046–2049.

Castori, M., Valente, E. M., Clementi, M., Tormene, A. P., Brancati, F., Caputo, V. & Dallapiccola, B. (2005) A novel locus for autosomal dominant cone and cone-rod dystrophies maps to the 6p gene cluster of retinal dystrophies. *Invest Ophthalmol Vis Sci* **46**, 3539–3544.

Colland, F., Jacq, X., Trouplin, V., Mougin, C., Groizeleau, C., Hamburger, A., Meil, A., Wojcik, J., Legrain, P. & Gauthier, J. M. (2004) Functional proteomics mapping of a human signaling pathway. *Genome Res* **14**, 1324–1332.

Cremers, F. P., van de Pol, D. J., van Driel, M., den Hollander, A. I., van Haren, F. J., Knoers, N. V., Tijmes, N., Bergen, A. A., Rohrschneider, K., Blankenagel, A., Pinckers, A. J., Deutman, A. F. & Hoyng, C. B. (1998) Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* **7**, 355–362.

Cui, J., Wang, W., Lai, M. D., Xu, E. P., Lv, B. J., Lin, J., Ruan, W. J., Ma, Y. & Yao, C. (2006) Identification of a novel VNTR polymorphism in C6orf37 and its association with colorectal cancer risk in Chinese population. *Clin Chim Acta* **368**, 155–159.

Deery, E. C., Vithana, E. N., Newbold, R. J., Gallon, V. A., Bhattacharya, S. S., Warren, M. J., Hunt, D. M. & Wilkie, S. E. (2002) Disease mechanism for retinitis pigmentosa (RP11) caused by mutations in the splicing factor gene PRPF31. *Hum Mol Genet* **11**, 3209–3219.

Derynck, R. & Zhang, Y. E. (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* **425**, 577–584.

Dharmaraj, S., Li, Y., Robitaille, J. M., Zhu, D., Mitchell, T. N., Maltby, L. P., Baffoe-Bonnie, A. B. & Maumenee, I. H. (2000) A novel locus for Leber congenital amaurosis maps to chromosome 6q. *Am J Hum Genet* **66**, 319–326.

George, A. L. Jr. (2004) Molecular basis of inherited epilepsy. *Arch Neurol* **61**, 473–478.

Guenther, E., Schmid, S., Hewig, B. & Kohler, K. (1996) Two-fold effect of Angiotensin II on voltage-dependent calcium currents in rat retinal ganglion cells. *Brain Res* **718**, 112–116.

Helmlinger, D., Hardy, S., Abou-Sleymane, G., Eberlin, A., Bowman, A. B., Gansmuller, A., Picaud, S., Zoghbi, H. Y., Trottier, Y., Tora, L. & Devys, D. (2006) Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. *PLoS Biol* **4**, e67.

Honjo, Y., Nagineni, C. N., Larsson, J., Nandula, S. R., Hooks, J. J., Chan, C. C., Karlsson, S. & Kulkarni, A. B. (2007) Neuron-specific TGF-beta signaling deficiency results in retinal detachment and cataracts in mice. *Biochem Biophys Res Commun* **352**, 418–422.

Inglehearn, C. F. (1998) Molecular genetics of human retinal dystrophies. *Eye* **12**, 571–579.

Jimenez-Sierra, J. M., Ogden, T. E. & van Boemel, G. B. (1989) *Inherited Retinal Diseases: A Diagnostic Guide*. St Louis, Mosby.

Kelsell, R. E., Godley, B. F., Evans, K., Tiffin, P. A., Gregory, C. Y., Plant, C., Moore, A. T., Bird, A. C. & Hunt, D. M. (1995) Localization of the gene for progressive bifocal chorioretinal atrophy (PBCRA) to chromosome 6q. *Hum Mol Genet* **4**, 1653–1656.

Kelsell, R. E., Gregory-Evans, K., Gregory-Evans, C. Y., Holder, G. E., Jay, M. R., Weber, B. H. F., Moore, A. T., Bird, A. C. & Hunt, D. M. (1998) Localization of a gene (CORD7) for a dominant

cone-rod dystrophy to chromosome 6q. *Am J Hum Genet* **63**, 274–279.
Lagali, P. S., Kakuk, L. E., Griesinger, I. B., Wong, P. W. & Ayyagari, R. (2002) Identification and characterization of C6orf37, a novel candidate human retinal disease gene on chromosome 6q14. *Biochem Biophys Res Commun* **293**, 356–365.
Li, Y., Marcos, I., Borrego, S., Yu, Z., Zhang, K. & Antinolo, G. (2001) Evaluation of the ELOVL4 gene in families with retinitis pigmentosa linked to the RP25 locus. *J Med Genet* **38**, 478–480.
Lim, J., Hao, T., Shaw, C., Patel, A. J., Szabo, G., Rual, J. F., Fisk, C. J., Li, N., Smolyar, A., Hill, D. E., Barabasi, A. L., Vidal, M. & Zoghbi, H. Y. (2006) A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. *Cell* **125**, 801–814.
Marcos, I., Borrego, S. & Antinolo, G. (2003) Molecular cloning and characterization of human RAB23, a member of the group of Rab GTPases. *Int J Mol Med* **12**, 983–987.
Marcos, I., Galan, J. J., Borrego, S. & Antinolo, G. (2002) Cloning, characterization, and chromosome mapping of the human GlcAT-S gene. *J Hum Genet* **47**, 677–680.
Marcos, I., Ruiz, A., Blaschak, C. J., Borrego, S., Cutting, G. R. & Antiñolo, G. (2000) Mutation analysis of GABRR1 and GABRR2 in autosomal recessive retinitis pigmentosa (RP25). *J Med Genet* **37**, E5.
Mathura, J. R., Jafari, N., Chang, J. T., Hackett, S. F., Wahlin, K. J., Della, N. G., Okamoto, N., Zack, D. J. & Campochiaro, P. A. (2000) Bone morphogenetic proteins-2 and -4: negative growth regulators in adult retinal pigmented epithelium. *Invest Ophthalmol Vis Sci* **41**, 592–600.
Nakamura, Y., Koyama, K. & Matsushima, M. (1998) VNTR (variable number of tandem repeat) sequences as transcriptional, translational, or functional regulators. *J Hum Genet* **43**, 149–152.
Romero, D. G., Plonczynski, M., Vergara, G. R., Gomez-Sanchez, E. P. & Gomez-Sanchez, C. E. (2004) Angiotensin II early regulated genes in H295R human adrenocortical cells. *Physiol Genomics* **19**, 106–116.
Ruiz, A., Borrego, S., Marcos, I. & Antiñolo, G. (1998) A major locus for autosomal recessive retinitis pigmentosa on 6q, determined by homozygosity mapping of chromosomal regions that contain γ-aminobutyric acid-receptor clusters. *Am J Hum Genet* **62**, 1452–1459.
Savaskan, E., Loffler, K. U., Meier, F., Muller-Spahn, F., Flammer, J. & Meyer, P. (2004) Immunohistochemical localization of angiotensin-converting enzyme, angiotensin II and AT1 receptor in human ocular tissues. *Ophthalmic Res* **36**, 312–320.
Schulz, H. L., Goetz, T., Kaschkoetoe, J. & Weber, B. H. (2004) The Retinome – Defining a reference transcriptome of the adult mammalian retina/retinal pigment epithelium. *BMC Genomics* **5**, 50.
Sehgal, R., Andres, D. J., Adler, R. & Belecky-Adams, T. L. (2006) Bone morphogenetic protein 7 increases chick photoreceptor outer segment initiation. *Invest Ophthalmol Vis Sci* **47**, 3625–3634.
Small, K. W., Weber, J. L., Roses, A., Lennon, F., Vance, J. M. & Pericak-Vance, M. A. (1992) North Carolina macular dystrophy is assigned to chromosome 6. *Genomics* **13**, 681–685.
Soeby, K., Larsen, S. A., Olsen, L., Rasmussen, H. B. & Werge, T. (2005) Serotonin Transporter: Evolution and Impact of Polymorphic Transcriptional Regulation. *Am J Med Genet B Neuropsychiatr Genet* **136B**, 53–57.
Stone, E. M., Nichols, B. E., Kimura, A. E., Weingeist, T. A., Drack, A. & Sheffield, V. C. (1994) Clinical features of a Stargardt-like dominant progressive macular dystrophy with genetic linkage to chromosome 6q. *Arch Ophthalmol* **112**, 765–772.
Verpy, E., Leibovici, M., Zwaenepoel, I., Liu, X. Z., Gal, A., Salem, N., Mansour, A., Blanchard, S., Kobayashi, I., Keats, B. J., Slim, R. & Petit, C. (2000) A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet* **26**, 51–55.
Wells, J., Wroblewski, J., Keen, J., Inglehearn, C., Jubb, C., Eckstein, A., Jay, M., Arden, G., Bhattacharya, S., Fitzke, F. & Bird, A. (1993) Mutations in the human retinal degeneration slow (*RDS*) gene can cause either retinitis pigmentosa or macular dystrophy. *Nat Genet* **3**, 213–218.
Yu, J., He, S., Friedman, J. S., Akimoto, M., Ghosh, D., Mears, A. J., Hicks, D. & Swaroop, A. (2004) Altered expression of genes of the Bmp/Smad and Wnt/calcium signaling pathways in the cone-only Nrl-/- mouse retina, revealed by gene profiling using custom cDNA microarrays. *J Biol Chem* **279**, 42211–42220.

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