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Exclusion of Four Candidate Genes, *KHDRBS2, PTP4A1, KIAA1411* and *OGFRL1,* as Causative of Autosomal Recessive Retinitis Pigmentosa

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Key Words
Retinitis pigmentosa · Mutation screening · Gene expression

Abstract
To identify the disease gene in 6 Spanish families with autosomal recessive retinitis pigmentosa linked to the RP25 locus, mutation screening of 4 candidate genes, *KHDRBS2*, *PTP4A1*, *KIAA1411* and *OGFRL1,* was undertaken based on their expression or functional relevance to the retina. Twenty-six single nucleotide polymorphisms were identified, of which 14 were novel. Even though no pathological mutations were detected, these genes however remain as good candidates for other retinal degenerations mapping to the same chromosomal region.

Introduction

Retinitis pigmentosa (RP, OMIM No. 268000) is a clinically and genetically heterogeneous group of retinal degenerations primarily affecting the rod photoreceptors. Diagnostic features include night blindness, attenuated retinal vessels, waxy pallor of the optic disc and retinal bone-spicule-like pigmentation. Autosomal recessive, autosomal dominant and X-linked modes of inheritance are common to RP. A complete set of genes and genetic loci associated with RP is documented in http://www.sph.uth.tmc.edu/retnet/. Here we report a mutation analysis of candidate genes in the RP25 locus which has previously been mapped in 6 Spanish families with recessive RP. The genetic interval spans approximately 16.1 cM between markers D6S257 and D6S1570 [1]. Subsequently, a three-generation consanguineous Pakistani family [2] also mapped within this interval (fig. 1).

To date, *GABRR1*, *GABRR2*, *ELOVL4*, *RAB23*, *GlcAT-S* and *RIM1* have all been excluded as the disease-causing gene for RP25 [3–7] (fig. 1). In order to identify the disease gene in our 6 Spanish families, candidate genes were selected on the basis of their function, tissue expression pattern and/or genetic data. Four interesting candidate genes – (hnRNP) K homology (KH) domain containing RNA binding signal transduction associated 2 *(KHDRBS2)*, protein tyrosine phosphatase type IV member 1 *(PTP4A1)*, hypothetical protein *(KIAA1411)* and opioid growth factor receptor-like 1 *(OGFRL1)* – were selected.

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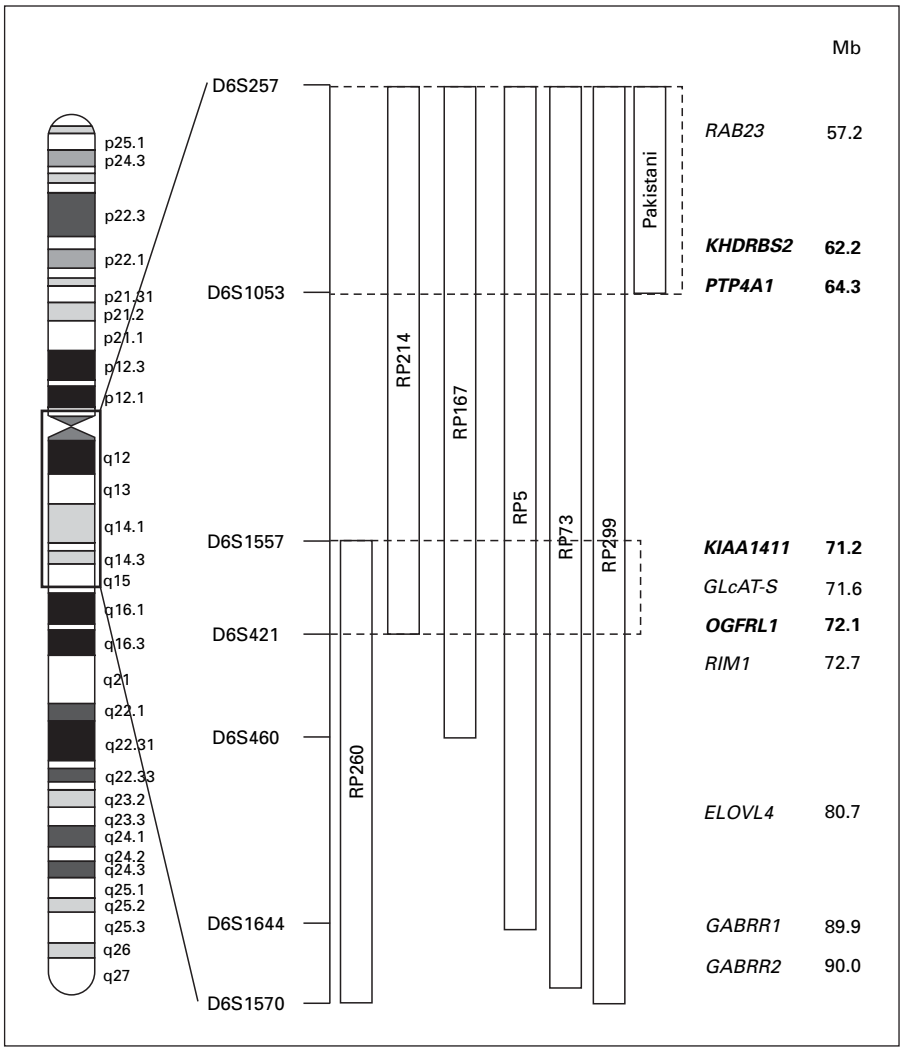

Fig. 1. The RP25 locus, the Pakistani locus and a common region of linkage in all Spanish families (between D6S1557 and D6S421). Genes in bold and normal fonts represent the genes that are screened in this work and that have been previously screened, respectively, with the physical distance (Mb) depicted alongside each gene.

It is possible that the Pakistani family and 5 of our Spanish families share a common gene or that the disease is caused by different genes in the two populations. In view of the first hypothesis, *KHDRBS2* and *PTP4A1* genes were selected from the region between D6S257 and D6S1053 where there is an overlap in the linkage data of both Spanish and Pakistani families (fig. 1). The other two genes, *KIAA1411* and *OGFRL,* were selected from the region between D6S1557 and D6S421 to which all the Spanish families were linked (fig. 1).

Materials and Methods

Informed consent was obtained from all participants for clinical and molecular genetic studies. The study conformed to the tenets of the declaration of Helsinki.

Genome sequences of the candidate genes were accessed through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), the Ensembl database (http://www.ensembl.org) and the UCSC human genome browser (http://genome.ucsc.edu/). To characterize exon-intron boundaries of the genes studied, the following program was employed (http://www.fruitfly.org/seq_tools/other.html). Information on expression patterns and expressed sequence tags were obtained from the National Centre for Biotechnology Information Unigene database. As *KIAA1411* has several alternatively spliced transcripts, one pair of primers was designed at exon 10 and exon 12 of *KIAA1411* and amplified in cDNAs from the retina, brain, kidney, liver, heart, skeletal muscle, pancreas, lung and placenta (Quick-Clone; Clontech). The PCR products were subjected to sequence analysis on the ABI 3100 automated sequencer.

Expression of all genes was assessed by PCR amplification of human cDNAs from the retina, brain, kidney, liver, heart, skeletal muscle, pancreas, lung and placenta (Quick-Clone; Clontech). Four pairs of primers were designed for the last exon and the 3′-

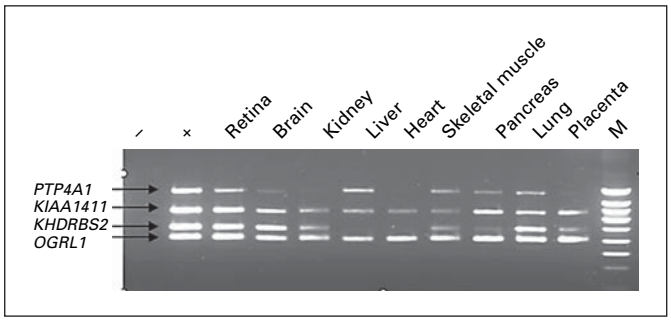

Fig. 2. Expression pattern of *PTP4A1*, *KIAA1411*, *KHDRBS2* and *OGFRL1* genes in human tissues as shown by multiplex PCR amplification. Lane 1: negative control; 2: positive genomic DNA control; lanes 3–11: cDNAs from retina, brain, kidney, liver, heart, skeletal muscle, pancreas, lung and placenta, respectively; M: 1-Kb smart ladder.

UTR of an individual gene to create gene-specific sequence-tagged sites.

Fifty pairs of primers were designed using Primer 3 Output (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) to screen the coding regions together with splice sites (GT/AG), and the 5′-UTR of the genes studied (primers are available on request). Full PCR conditions and the sequencing protocol are as described previously [8].

Results

The results of bioinformatics analysis of *KHDRBS2*, *PTP4A1* and *OGFRL1* conformed to the previously published data [9–11]. The results of BLAST analysis however showed that there is an additional exon (10A) between exon 10 and 11 in the KIAA1411-001 Ensembl transcript. To confirm the result of the BLAST analysis, a region between exon 10 and 12 was amplified in cDNAs from retina and various other tissues and sequenced. The results of sequence analysis showed that exon 10A which is 78 bp exists in the retina and other tissues. Based on this information, the transcript and translation lengths of *KIAA1411* will be 6,280 bp and 1,541 amino acids, respectively.

Expression analysis showed that all genes were expressed in the retina, brain, skeletal muscle, pancreas and lung. However, *PTP4A1* was expressed at a low level in the kidney and was not detected in either the heart or placenta. Meanwhile, *KHDRBS2* was not expressed in either the liver or heart (fig. 2).

Mutation screening of the *KHDRBS2* gene led to the identification of 10 genetic variants, of which 5 were novel (table 1). The 5′-UTR change was very interesting because it was heterozygous in one parent and homozygous in his affected son in a consanguineous family. Moreover, the same change was detected in a carrier state in an affected individual in another non-consanguineous family. However, it was detected with an allele frequency of 28% in 100 control chromosomes (table 1).

Mutation screening of *PTP4A1* showed no pathological or non-pathological changes.

Twelve single nucleotide changes were identified in *KIAA1411* (table 1), 2 of which were not detected in 200 Spanish control chromosomes and were segregating with the disease phenotype in 2 families. Of these 2 changes, 1 is a heterozygous missense change (N905H) and was detected in a non-consanguineous family. The other change is a homozygous silent change (L282L) in a consanguineous family. Although the mutation in the first family is expected to be compound heterozygous, we could not find the second change by screening all the coding exons and the 5′-UTR of the gene. Meanwhile, the homozygous L282L in the consanguineous family is a silent change.

Mutation screening of the *OGFRL1* gene revealed 2 novel changes, a deletion of an amino acid (lysine) from exon 7 and a novel non-conservative substitution at codon 47, which was detected in an unaffected member from all the studied families. The other 2 changes were intronic polymorphisms (table 1).

In total, 26 single nucleotide polymorphisms (SNPs) were identified, of which 14 were novel. Twelve of the 14 novel SNPs were identified in unaffected members of the families and/or in 100 control chromosomes. Only 2 changes in the *KIAA1411* gene were segregating with the disease phenotype and were not detected in 200 control chromosomes; however, they may represent very rare SNPs. All changes were assigned a nucleotide number starting at the first translation base of *KHDRBS2*, *KIAA1411* and *OGFRL1* according to the Genbank entries NM_152688, NM_020819 and NM_024576, respectively.

Discussion

Herein we report the screening of 4 candidate genes for mutations in 6 Spanish families that have been linked to the RP25 locus. The expression pattern of the genes using cDNA from different tissues including retina was evaluated. In addition, the genomic structure of the genes was confirmed through bioinformatics analyses.

Table 1. Changes detected in *KHDRBS2*, *KIAA1411* and *OGFRL1* genes

Gene	Sequence variation	AA change	Allele frequency, %	Published in SNP database or not
KHDRBS2	–67 A → C	–	28	not published
	IVS2-88 G → A	–		rs9346001
	c. 489 G → A	Q70Q		rs6921170
	IVS4_71delT	–	16.6	not published
	c. 1155 T → C	Y292Y		rs10484690
	IVS8+33 C → A	–		rs1555167
	c. 1277 C → T	P333L	8.3	not published
	c. 1281 G → A	Q334Q		rs1204114
	*72 G → T	–	83.3	not published
	*78 T → C	–	83.3	not published
KIAA1411	IVS3-15 C → T	–	50	not published
	c. 494 T → A	I30I		not published
	IVS8-27 A → G	–		rs2273210
	c. 1250 A → G	L282L	0	not published
	IVS12-196_201delTTTATA	–	50	not published
	c. 3117 A → C	N905H	0	not published
	c. 3833 C → T	N1143N		rs774087
	c. 4129 A → G	D1242G		rs2747701
	IVS16+23 A → G	–	16.6	not published
	IVS17-108 A → G	–		rs9283835
	IVS19+92 G → A	–	33.3	not published
	*8 G → A	–	41.6	not published
OGFRL1	c. 268 T → C	S47P	8.3	not published
	IVS4-11 A → T	–		rs2273889
	IVS6-3 C → T	–		rs16880821
	c. 1315_1317delAAG	deletion of lysine	4.7	not published

SNP = Single nucleotide polymorphism; c. = cDNA sequence. The asterisk denotes a change in the 3′-untranslated region.

Due to the absence of retina-specific genes in our region, we selected the candidates that are retina localized. All genes studied were ubiquitously expressed; however, they were also expressed in the retina, as determined by PCR amplification of retinal cDNA (fig. 2).

According to the genetic data obtained from linkage analysis, there is a chance that both the Pakistani family and 5 of our Spanish families could have a common gene. In agreement with this hypothesis, the *KHDRBS2* and *PTP4A1* genes were selected as the best candidates from the region between D6S257 and D6S1053 (fig. 1). *KHDRBS2*, the first gene to be selected from the top region between D6S257 and D6S1053, spanned approximately 600 kb. The gene was isolated during the large-scale sequencing analysis of the human fetal brain cDNA library and contained the KH domain which is embedded in a larger conserved domain called STAR domain. In this STAR domain, the protein had a 99% sequence identity with rat SLM-1 (Sam68-like mammalian protein 1) and 98% with mouse SLM-1 [9]. Moreover, the STAR family genes were conserved from yeast to human [12]. It has been postulated that this gene might have some functional homology with protein tyrosine kinases which has a role in photoreceptor degeneration [9]. For example *MERTK* which is the causative gene for autosomal recessive RP encodes a tyrosine kinase receptor and causes retinal degeneration through defective phagocytosis of shed photoreceptor outer segments by the retinal pigment epithelium [13]. The second selected gene from the top region, *PTP4A1*, encoded a unique nuclear protein tyrosine phosphatase which has a fundamental role in the regulation of cellular, physiological and pathogenic processes [14]. It is known that protein phosphorylation is crucial for the process of pre-mRNA splicing [15]. For

example, the mutant protein of *PAP-1*, the gene underlying the RP9 form of autosomal dominant RP, was accumulated in a dephosphorylated form in the cells of the nuclear matrix suggesting that phosphorylation of *PAP-1* is essential for normal function [16]. Furthermore, the human *PTP4A1* had 100% conservation at the amino acid level with the rat and mouse, and 55% amino acid identity with the *Caenorhabditis elegans* orthologues which suggests that the gene has a critical function in cellular regulation.

Assuming that all Spanish families may have the same gene which is different from the disease gene of the Pakistani family, we searched the region between D6S1557 and D6S421 for other candidate genes (fig. 1). This disease interval spans approximately 1 Mb and it contains 6 candidate genes; one of them has already been excluded (*GlcAT-S;* fig. 1) [5]. The remaining 5 genes were *COL9A1*, *KIAA1411*, *C6orf57*, *SMAP1* and *OGFRL1*, from which *KIAA1411* and *OGFRL1* were selected. *KIAA1411*, a novel hypothetical protein, is localized at 71.2 Mb between D6S1557 and D6S421 on chromosome 6. This gene in humans shows approximately 84 and 82% identity at the nucleic acid level with the rat and mouse orthologue, respectively, suggesting a conserved function of this protein in both organisms. The fourth gene, *OGFRL1*, spans approximately 13 kb of genomic DNA and is localized at 72 Mb from the telomere of the long arm of chromosome 6 (6q13). The gene has an OGFr conserved domain which is similar to the domain of the *OGFR* gene localized at 20q13.33. It has been postulated that opioid peptides act as growth factors in neural and non-neural cells and tissues, in addition to their role as neurotransmitters/neuromodulators [11].

The absence of pathogenic changes in the screened genes excluded them as the causative genes for the RP25 locus. However, we could not exclude these genes as good candidates for other retinal degenerations mapping to the same chromosomal region. The examination of other genes as candidates for RP25 is under way.

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