A novel missense mutation of the *GRK1* gene in Oguchi disease

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Abstract. Oguchi disease is a rare form of congenital stationary night blindness with an autosomal recessive inheritance pattern. The presence of S-antigen (*SAG*) and G-protein-dependent receptor kinase 1 (*GRK1*) mutations were investigated in the family members with Oguchi disease. All exons of the *SAG* and *GRK1* genes were amplified by polymerase chain reaction and sequenced. The patients were shown to have characteristic clinical features of Oguchi disease. Gene analysis determined a novel *GRK1* mutation c.923T>C, which caused Oguchi disease in all siblings. This mutation, was demonstrated by amino acid alignment analysis to be in a phylogenetically conserved region and resulted in an amino acid change from leucine to proline at position 308. Thus, the present study reports a novel missense mutation of *GRK1* in the affected members of a consanguineous Turkish family. Homozygosity at position 308, which resides in the catalytic domain of the *GRK1* gene, is the cause of Oguchi disease in this Turkish family.

Introduction

Congenital stationary night blindness (CSNB) is a group of disorders characterized by non-progressive retinal dysfunction (1). CSNB is caused by mutations in the phototransduction component or mutations in retinal signaling from the outer retina to the inner retina (1). There are two main types of CSNB with specific fundus findings, fundus albipunctatus and Oguchi disease. These disorders are inherited in an autosomal recessive pattern (2,3). A golden-brown or diffuse gray-white fundus discoloration is observed in Oguchi disease in the light adapted state. Following complete dark adaptation, the fundus returns to normal (1,2). Oguchi disease is caused by the mutations in G-protein-dependent receptor kinase 1 (*GRK1)* (MIM:180381) and S-antigen (*SAG)* (MIM:181031) (4). Patients are divided into two groups type 1 or type 2 based on mutations in two genes, namely, *SAG* and *GRK1*, respectively (2).

The aim of this study was to investigate the presence of these mutations, yet to be described in a Turkish population, in family members with newly diagnosed Oguchi disease.

Materials and methods

Family history. Four family members with night blindness were referred to the Ulucanlar Eye Hospital Retina Clinic (Ankara, Turkey). Four siblings: A 12-year-old male (case 1), 14-year-old female (case 2), 16-year-old female (case 3), 19-year-old female (case 4), and their 41-year-old mother (case 5) and 44-year-old father (case 6) were examined. Cases 1-4 suffered night blindness since early childhood. The mother and father were 4th degree relatives (first cousins) and thus had a consanguineous marriage. None of the cases had diabetes, hypertension, a history of systemic or ocular disease, nor had any undergone any surgery.

A complete ophthalmological examination was conducted including visual acuity, intraocular pressure measurement with Goldmann applanation tonometry, biomicroscobic examination and dilated pupil examination of the posterior segment.

Ethical approval. Study procedures were conducted in accordance with the Declaration of Helsinki. The study protocol was approved by The Ethical Committee of Diskapi Training and Research Hospital (Ankara, Turkey) and written informed content was obtained from all study participants. All patients were Turkish Caucasians. This study is registered as an Australian New Zealand Clinical Trials Registry (no. ACTRN 368991).

Mutation testing and Sanger sequencing. All six family members underwent mutation testing. Whole blood (10 ml) was taken from each family member. Genomic DNA was obtained from peripheral leukocytes by ammonium acetate extraction (AppliChem GmbH, Gatersleben, Germany). To conduct Sanger sequencing, DNA samples were quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, NC, USA) prior to polymerase chain reaction (PCR). Primer sequences (Table I) used for PCR reactions were determined manually by using Ensembl Database ID's ENST00000335678 (*GRK1*) and ENST00000409110 (*SAG*).

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Table I. Primer details for polymerase chain reaction.

A, *SAG* primer details: ENSEMBL: ENST00000409110

Exon	Amplicon size (bp)	Annealing temperature (˚C)	Primer sequences
Exon2	276	54	SAG-E2F: 5'-GGATCTCGTGAGTAGGTTTC-3' SAG-E2R: 5'-CACTGTACTTGAAAAAGCTCC-3'
Exon3	294	60	SAG-E3F: 5'-CATATTGGCCAGGCTCAAAC-3' SAG-E3R: 5'-AAAGTGAGCGGTTATCTGTGAC-3'
Exon4	330	54	SAG-E4F: 5'-AATGAACATGGATTACATGTG-3' SAG-E4R: 5'-GTCACGTGAATTAGGTACAGG-3'
Exon5	342	63	SAG-E5F: 5'-GGTTGAAAACCCGTGTTCGC-3' SAG-E5R: 5'-CACATGATAAGGTGCTGCGG-3'
Exon6	218	60	SAG-E6F: 5'-TAATGGAACAGCCCCTTCTG-3' SAG-E6R: 5'-CCCAGCATTGGTGACAGAGT-3'
Exon7	319	60	SAG-E7F: 5'-TGCAACCCCGAATAGGACAT-3' SAG-E7R: 5'-CAGCCCTATGGGAAGAGGTCT-3'
Exon8	359	60	SAG-E8F: 5'-GAGCATTCCTGGAGAATCTCC-3' SAG-E8R: 5'-GAAACAAGCTTCCTTGCAAGG-3'
Exon9	336	60	SAG-E9F: 5'-GTGTTTCAGGCCCTTCCTTAG-3' SAG-E9R: 5'-CAGACCAGAGAAGTGACCTCTC-3'
Exon10	431	60	SAG-E10F: 5'-ACAGGACTTCAAAACCCCAG-3' SAG-E10R: 5'-GGTGTGGTAGATGCAGAGCTAAG-3'
Exon11	360	60	SAG-E11F: 5'-GTCAAGTTCCCAGGCTCTTG-3' SAG-E11R: 5'-CAGGGTGATGTGAAGGGAAG-3'
Exon12	226	60	SAG-E12F: 5'-CTGCCCATCTGCTCTTCACC-3' SAG-E12R: 5'-CTCCCAGTCATTCAGGAAAGG-3'
Exon13	252	60	SAG-E13F: 5'-GATGTTGTGAGTTCGGGTGC-3' SAG-E13R: 5'-CACAACTGTCCAGAAAGCAGC-3'
Exon14	248	63	SAG-E14F: 5'-TGTGACTCTCCGCAGCCATAG-3' SAG-E14R: 5'-CACTCCCATGCTCTGAGATGC-3'
Exon15	251	60	SAG-E15F: 5'-ACGCAGTGATCATGAACTGC-3' SAG-E15R: 5'-GACTCAAAGAGGGTTTTGTGC-3'
Exon16	348	63	SAG-E16F: 5'-CCTTGATCAGTTCCTTCGTTGC-3' SAG-E16R: 5'-CCAGGGGAGAACAAACAAGCT-3'

B, GRK1 primer details: ENSEMBL: ENST00000335678

Exon	Amplicon size (bp)	Annealing temperature (˚C)	Primer sequences
Exon1/1	443	64	GRK1-E1/1F: 5'-TGCTCTGTCTGTGAACGCTCC-3' GRK1-E1/1R: 5'-AGAAGAGTTTGGCCTGGGGG-3'
Exon1/2	526	64	GRK1-E1/2F: 5'-CGGCAGACAATGACCTCCAG-3' GRK1-E1/2R: 5'-AGGCACCAGCTGTTAAGGGC-3'
Exon2	280	62	GRK1-E2F: 5'-CGATGCACCTAGTCCCTTTCC-3' GRK1-E2R: 5'-ATGGCTCTGCCTGTGGAAAG-3'
Exon3	360	62	GRK1-E3F: 5'-TCAAAACGACCAGAACGCTG-3' GRK1-E3R: 5'-TCGTGAGGTTGTGCAGAGACC-3'
Exon4	207	64	GRK1-E4F: 5'-TGTGCAGCCAGGGGTGACTC-3' GRK1-E4R:5'-GTATGTGCAAGTGCACACAGGC-3'
Exon5	291	62	GRK1-E5F: 5'-AGCATCAGTCCTGCGATTCC-3' GRK1-E5R: 5'-CAGTAACGATCCCATCACTGCC-3'
Exon6	353	62	GRK1-E6F: 5'-TCTGGTCTGACCACCCAAGAG-3' GRK1-E6R: 5'-CCGACTCTCACAGGCTGGAC-3'
Exon7	451	64	GRK1-E7F: 5'-GGCTAAACGGCGCTTCCTTC-3'

SAG, S-antigen; *GRK1*, G-protein-dependent receptor kinase 1.

Table II. Amino acid alterations in *SAG* and *GRK1* genes.

A, SAG amino acid alterations

Case	Gene region	Alteration	Condition	SNP ID	Amino acid alterations
1	Exon 16	c.1207G>A	Heterozygote	rs1046974	Val403Ile
2	Exon 16	c.1207G>A	Homozygote	rs1046974	Val403Ile
	Intron 4-5	c.181+82A>G	Homozygote	rs2304777	-
	Intron 6-7	c.436-18G>C	Homozygote	rs2304774	-
	Intron 9-10	c.733+31T>G	Homozygote	rs745498	-
3	Exon 16	c.1207G>A	Homozygote	rs1046974	Val403Ile
4	Exon 16	c.1207G>A	Heterozygote	rs1046974	Val403Ile
5	Exon 16	c.1207G>A	Homozygote	rs1046974	Val403Ile
6	Exon 16	c.1207G>A	Heterozygote	rs1046974	Val403Ile

B, GRK1 amino acid alterations

Case	Gene region	Alteration	Condition	SNP ID	Amino acid alterations
1	Exon 3	c.923T>C	Homozygote	Novel	Leu308Pro
2	Exon 3	c.923T>C	Homozygote	Novel	Leu308Pro
3	Exon 3	c.923T>C	Homozygote	Novel	Leu308Pro
4	Exon 3	c.923T>C	Homozygote	Novel	Leu308Pro
5	Exon 3	c.923T>C	Heterozygote	Novel	Leu308Pro
6	Exon 3	c.923T>C	Heterozygote	Novel	Leu308Pro

SAG, S-antigen; *GRK1*, G-protein-dependent receptor kinase 1; SNP, single nucleotide polymorphism.

Then 200 ng genomic DNA was combined with 5X Buffer (Promega Corporation, Madison, WI, USA), 0.2 mM of each dNTP (Promega Corporation), 1.5 mM $MgCl_2$ (Promega Corporation), 0.2 μM of forward and reverse primers (IDT, Inc., Coralville, IA, USA), 1 unit GoTaq DNA Polymerase (Promega Corporation) and nuclease-free water up to 25 μl. The PCR mix was vortex mixed, centrifuged at 650 x g for 1 min, then transferred to a thermal cycler (ABI 9700; Applied Biosystems, Foster City, CA, USA). Samples were amplified with an initial heat denaturation step of 94˚C for 5 min; followed by 35 cycles of 94˚C for 30 sec, 54-65˚C for 30 sec, and 72˚C for 30 sec, and a final of extension at 72˚C for 7 min. Primer sequences and annealing temperatures are shown in Table I. PCR products were run on agarose gels and samples with the correct band size were purified using Wizard SV Gel and PCR Cleanup system (Promega Corporation, Madison, WI, USA). After purification, PCR products were cycle sequenced using forward primers and the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Inc.). After cycle sequencing, products were cleaned up using ZR DNA Sequencing Clean up kit (Zymo Research, Irvine, CA, USA) and loaded onto 96-well plates of a 3130 Genetic Analyzer (Applied Biosystems). Analysis of the results was conducted using SeqScape 3 Software (Applied Biosystems).

Bioinformatics analysis. Two of the most popular bioinformatics tools [Sorting Intolerant from Tolerant (SIFT; http://sift.bii.a-star.edu.sg/) and Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/)] were used to predict whether the amino acid substitutions identified in the study affect protein function. These types of tools use predictions based on the degree of conservation of amino acid residues in sequence alignments derived from closely associated sequences, and additionally the impact of the substitution on the structure and function of the protein.

Results

Patient characteristics. The family members (four siblings 12-year-old male, 14-year-old female, 16-year-old female and 19-year-old, and their 41-year-old mother and a 44-year-old father were examined. There was no history of night blindness in either the mother or the father. However, four of the family members had a history of nyctalopia since early childhood. Their visual acuity was 20/20 bilaterally and they had no refractive error. All children and their parents had normal intraocular eye pressure (11-19 mmHg) values. All patient's eyes were phakic and slit-lamp examination revealed normal findings. Fundus examination of parents revealed normal findings. However, fundus images of the four children showed a characteristic golden metallic reflex in all areas of the fundus. The Mizuo-Nakamura phenomenon was demonstrated: The fundus color changed to normal after three hours of dark adaptation.

Sanger sequencing results. Sanger sequencing for two candidate genes, *GRK1* and *SAG* was conducted. The coding regions

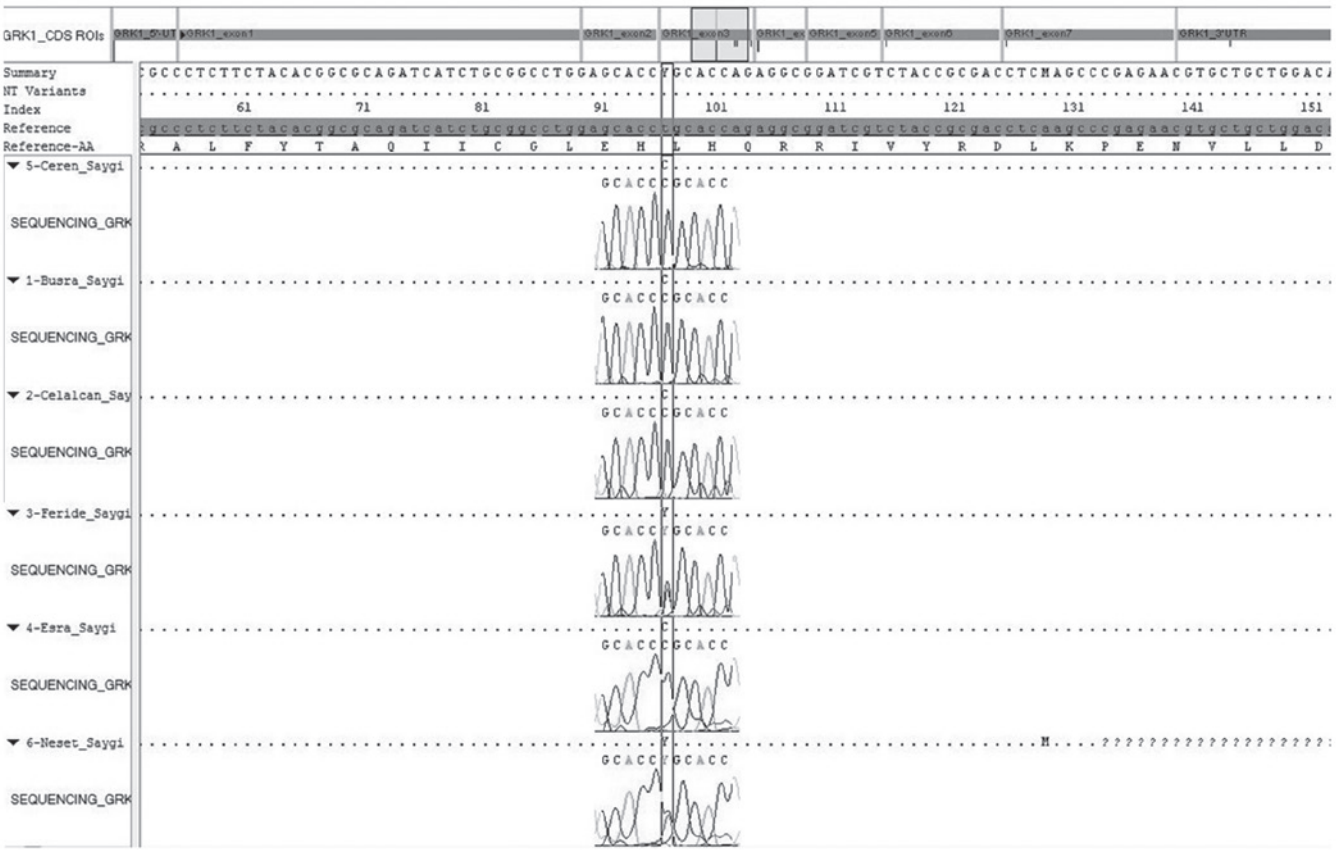
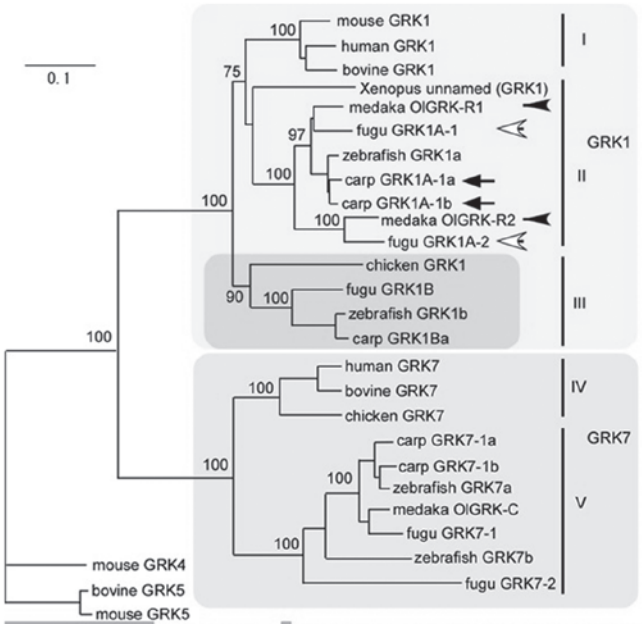


Figure 1. Partial nucleotide sequences of exon 3 of the family. A novel homozygous variation (c.923T>C) is highlighted, resulting in the substitution of leucine (CTG) for proline (CCG) at position 308. Parents were heterozygous for this mutation.

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Human GRK1    300 QIICGLEHLHQRRIVYRDLKPENVLLDNDGNVRISDLGLAVELLDGQSKTKGYAGTPGFMAPELLQGEEYDFSVDYFALGVTLYEMIAARGPFRARGEKV  399
Mouse GRK1    300 QIISGLEHLHQRRIVYRDLKPENVLLDNDGNIRISDLGLAVELKEGQNKTKGYAGTPGFMAPELLRGEEYDFSVDYFALGVTLYEMIAARGPFRARGEKV  399
Bovine GRK1   297 QIVSGLEHLHQRNIIYRDLKPENVLLDDDGNVRISDLGLAVELKAGQTKTKGYAGTPGFMAPELLLGEEYDFSVDYFALGVTLYEMIAARGPFRARGEKV  396
Fugu GRK1A-1  301 QIIQGLEHLHQRRIIYRDLKPENVLLDNEGNVRISDLGLAVELADHQDKIKGYAGTPGFMAPELLKGEEYDYSVDYFALGVTVYEFIAAKGPFRTRGEKV  400
Carp GRK1A-1a 301 QIIQGLEHLHQKRIIYRDLKPENVLLDNEGHVRISDLGLAVELADDQLKTKGYAGTPGFMAPELLKGEEYDYSVDYFTLGVTLYEFIAAKGPFRTRGEKV  400
Carp GRK1A-1b 301 QIIQGIEHLHQKRIIYRDLKPENVLLDNEGNVRISDLGLAVELADDQLKTKGYAGTPGFMAPELLKGEEYDYSVDYFTLGVTLYEFIAAKGPFRTRGEKV  400
Fugu GRK1A-2  787 QIIQGLEHLHQKRIIYRDLKPENVLLDNDGNVRISDLGLAVELKEGKTMTKGYAGTPGYMPPEMLKGEKYDSCVDYFSLGVTLFEFLAAKNPFRNRGEKV  886
Fugu GRK1B    300 QIICGLEHLHQHRIVYRDLKPENVLLDDAGHVRLSDLGLAVELPPGKDKTSGYAGTPGFMAPELLEKKEYDYTVDYFTLGVTLYEMIAAKGPFRVRGEKV  399
Carp GRK1Ba   300 QIICGLQHLHQHRIVYRDLKPENVLLDDAGHVRLSDLGLAVELPPGKDKTQGYAGTPGFMAPELLQKKEYDYSVDYFTLGVTLYEMVAAKGPFRCRGEQV  399
Carp GRK1Bb    35 QIICGLEHLHQHRIVYRDLKPENVLLDDAGHVRLSDLGLAVELPPGKDKTQGYAGTPGFMAPELLQKKEYDYTVDYFTLGVTLYEMVAAKGPFRCRGEQV 1134
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Figure 2. Molecular phylogenetic tree of vertebrate *GRK1* genes. Branch length is proportional to the number of amino acid substitutions; the scale bar represents 0.1 amino acid substitutions per position in the sequences.

and their flanking sequences were analyzed in the 4 siblings and their parents to identify mutations that may be causing Oguchi disease. Sequencing results were analyzed with SeqScape 3 software (Applied Biosystems) by aligning to the Human Reference Genome Sequence (GRCh38/hg38), and single nucleotide variations (SNVs) were determined. The single nucleotide polymorphism (SNP) database dbSNP (build 141) served as a reference for registered SNPs, and non-synonymous SNVs were extracted. Heterozygous and homozygous variations were identified, including *de novo* heterozygous variations and a homozygous variation, as presented in Table II. Molecular analysis of the *SAG* gene revealed no SNPs that segregated with the disease. The mutation analysis of the other candidate gene, *GRK1*, demonstrated a novel mutation (c.923T>C) (Fig. 1), shared by all family members, which was homozygous in all siblings and heterozygous in the two parents, consistent with autosomal recessive transmission (Table II). This novel finding directed us to determine whether this position is conserved phylogenetically among other *GRK1* orthologs by utilizing Clustal W2.1 (Conway Institute UCD, Dublin, Ireland). Nucleotide changes resulting in the change of the strictly conserved residue 308 from leucine to proline affects the protein kinase catalytic domain of the gene. Popular bioinformatics tools SIFT and PolyPhen-2 analysis scores (0 and 1.000, respectively) confirm this change to be damaging. Further analysis is required in healthy populations to confirm pathogenicity. No other mutations were found in the *GRK1* gene in the patients. The molecular phylogenetic tree of vertebrate *GRK1* genes, which are highly conserved, is shown in Fig. 2. Branch length is proportional to the number of amino acid substitutions; the scale bar represents 0.1 amino acid substitutions per position in the sequences. As the branches are short, we can therefore predict damaging effects from the occurrence of amino acid changes (5).

Discussion

CSNB caused by defective signaling from photoreceptors to bipolar cells is characterized by a reduced or absent b-wave and a normal a-wave in electroretinography (6). Oguchi disease is an uncommon form of CSNB, characterized by specific clinical characteristics, termed the Mizuo-Nakamura phenomenon (7). Two genes are known to be involved in Oguchi disease: *SAG* and *GRK1* gene (7). In a European study, all the reported patients had mutations in *GRK1*. However, the majority of Japanese patients with Oguchi disease have another causative gene, *SAG* (8).

SAG, also termed arrestin, encodes a major protein of the outer retinal segment. It binds to activated rhodopsin in retinal rod outer segments. Mutations in this gene have previously been associated with Oguchi disease (9). In the present study a novel missense mutation was identified.

The *GRK1* gene encodes rhodopsin kinase, responsible for the phosphorylated rhodopsin in rod cells (10). Mutations in *GRK1* can result in the Oguchi phenotype. This study demonstrated that a novel amino acid change (p.Leu308Pro) was common in all siblings in the family in a homozygous state and thus may have resulted in Oguchi disease.

To date, in the Turkish population there have been no reports of *GRK1* mutations causing Oguchi disease. In the present study, a novel nonsense mutation in *GRK1* in affected members of a consanguineous Turkish family was identified. In conclusion, a novel *GRK1* variant (c.923T>C) was shown the be the cause of Oguchi disease in 4 family members who inherited the mutation from a common ancestor of their parents.

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