

A novel *HSF4* gene mutation (p.R405X) causing autosomal recessive congenital cataracts in a large consanguineous Pakistani family

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Abstract

Background: Hereditary cataracts are most frequently inherited as autosomal dominant traits, but can also be inherited in an autosomal recessive or X-linked fashion. To date, 12 loci have been mapped for autosomal recessive cataracts including one locus on chromosome 16q22 containing the disease causing gene *HSF4*. Here, we describe a Pakistani family with the first nonsense mutation in *HSF4* thus expanding the mutational spectrum of this heat shock transcription factor gene.

Methods: A large consanguineous Pakistani family with autosomal recessive cataracts was collected from Quetta. Genetic linkage analysis was performed for all known autosomal recessive cataracts loci and linkage to a locus containing *HSF4* (OMIM 602438) was found. All exons and splice sites of the heat shock transcription factor 4 (*HSF4*) were sequenced. A mutation specific restriction enzyme digest (*HphI*) was performed for all family members.

Results: The disease phenotype perfectly co-segregated with markers flanking the known cataract gene *HSF4*. A maximum two-point LOD score with a $Z_{max} = 5.6$ at $\theta = 0$ was obtained for *D16S421*. Direct sequencing of *HSF4* revealed the nucleotide exchange c.1213C>T in this family predicting an arginine to stop codon exchange (p.R405X).

Conclusion: Our findings identified the first nonsense mutation (p.R405X) in exon 11 of *HSF4* in a large consanguineous Pakistani family with autosomal recessive cataract.

Background

Congenital cataracts show considerable clinical and locus heterogeneity and are one of the major causes of vision loss in world-wide[1,2]. Cataracts can be isolated or can occur in association with a large number of metabolic diseases and genetic syndromes [3].

Nonsyndromic congenital cataracts tend to be highly penetrant as Mendelian traits with autosomal dominant more common than autosomal recessive forms. Nonsyndromic congenital cataract has an estimated frequency of 1-6 per 100,000 live births [4]. To date, loci for 28 autosomal dominant and 12 autosomal recessive forms of congenital cataracts have been mapped. Out of 12 autosomal recessive loci, mutations in eight genes have been identified [5-16]. Of the cataracts families for whom the mutant gene is known, about half show mutations in crystallins, about a quarter have mutations in connexins, with the remainder divided among the genes of the heat shock transcription factor-4 (*HSF4*), aquaporin-0 (*AQP0*, MIP), and beaded filaments structural proteins-2 (*BFSP2*) [3].

Here, we report a large consanguineous Pakistani family with eight affected individuals having autosomal recessive cataracts. Consanguineous marriages are a common practice in Pakistani society and 60 % marriages are reported within families [17]. These families are instrumental for mapping disease loci and for identification of causative genes and mutations. During linkage analysis for known recessive cataracts loci, we showed complete co-segregation of the disease phenotype with markers *D16S397* (85.94 cM), *D16S3086* (85.94 cM) and *D16S421* (85.94 cM). A maximum two-point LOD score of 5.6 at recombination fraction $\theta = 0$ was obtained for marker *D16S421*. As the critical

interval contained the *HSF4* gene previously reported to cause autosomal dominant or autosomal recessive cataracts, sequence analysis of *HSF4* was performed which identified a novel nonsense mutation (p.R405X) in exon 11 as the cause of the disease in this family.

Methods

Family enrollment and clinical evaluation

The family (BUIT-CA01) was enrolled from Layton Rahmatullah Benevolent Trust (LRBT) Hospital Quetta, Pakistan. Approval for this study was obtained from the IRB at Faculty of Biotechnology and Informatics, BUITEMS, Quetta Pakistan. Written informed consent was obtained from all the participating subjects or their guardians with the tenets of the Declaration of Helsinki. A detailed medical history was obtained by interviewing all the family members. Cataracts in affected individuals were either present at birth or developed during infancy. All the affected individuals had undergone cataract surgery so the lens phenotype could not be ascertained in more detail. During clinical examinations of affected individuals (IV:1, IV:2, IV:3, IV:4, IV:5, IV:6, IV:7 and IV:8), there was no other ocular or systematic abnormalities except cataracts. Neither of the parents (III:1, III:2, III:4 and III:5) and none of the unaffected individuals (II:4 and III:3) has any evidence of cataracts.

Linkage analysis

Genomic DNA was extracted from blood samples obtained from all participating family members by a standard protocol [18]. All reported autosomal recessive cataract loci were

genotyped by selecting at least three STR markers for each candidate interval. Thermal cycling was performed with an initial activation step at 94 °C for 3 min following by 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 40 s with a final extension at 72 °C for 10 min. Amplified product was run on 6% denaturing polyacrylamide gel and was visualized by silver staining. For linkage analysis, the Marshfield genetic map (<http://research.marshfieldclinic.org/>) was consulted for marker order and map distances. Two-point Lod scores were calculated by using 2-point linkage analysis with the FASTLINK program in the easyLINKAGE software package [19]. The disease was coded as fully penetrant and the disease allele frequency was set at 0.0001. Meiotic recombination frequencies were assumed to be equal for males and females.

Mutational Analysis

Primers for PCR amplification and subsequent sequencing of *HSF4* were designed by using software at the primer3 web site (<http://frodo.wi.mit.edu/cgi-bin/primer3/www.cgi>) to flank all exon-intron boundaries. Exons amplification, sequencing reactions and mutational analysis were performed by standard protocols. DNA sequencing was performed using BigDye version 2.1 and an ABI 3100 sequencing apparatus (Applied Biosystem, Foster City, CA). All exons and exon/intron border regions were sequenced and aligned to the GenBank reference sequences. Reactions were performed in 10 ul volumes according to the manufactures' protocols. PCR reactions were analyzed by 2% agarose gel electrophoresis by staining with ethidiumbromide before sequencing. Thermal cycling conditions were an initial activation step at 95 °C for 5 min following by

first 10 cycles as touch down PCR (with annealing 68 °C to 58 °C) and additional 27 cycles with denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The identified mutation was analyzed by an allele-specific restriction enzyme (*HphI*) digest. Restriction enzyme digest was performed in a reaction volume of 20 ul, using 4 ul PCR product and 5 units of the enzyme. The digested PCR products were analyzed by 2% agarose, 1X TBE, and DNA visualized by staining with ethidiumbromide.

Results

This family (Fig. 1) originated from Afghanistan and migrated to Quetta-Pakistan in the late 1980s. Cataracts in affected individuals were either present at birth or developed during infancy. By locus-specific linkage analysis, the disease phenotype was shown to perfectly co-segregate with flanking markers to the already known causative gene *HSF4* (OMIM 602438). A highly significant maximum two-point LOD score was obtained at *D16S421* $Z_{max} = 5.6$ at $\theta = 0$ (assuming equal allele frequencies). PCR amplification and subsequent direct sequencing of *HSF4* revealed the novel nucleotide exchange c.1213C>T in this family (Fig. 2). This mutation was not found in any of the unaffected individuals of this family. This transition is predicted to change the amino acid arginine into a stop codon (p.Arg405X) (Fig 2). The mutation-specific restriction enzyme (*HphI*) digestion confirmed homozygosity for the mutant allele in all eight affected individuals (IV:1, IV:2, IV:3, IV:4, IV:5, IV:6, IV:7 and IV:8; bands of 403 bp, 245 bp and 148 bp) whereas homozygous wild type alleles were shown in individual II:4 and III:3 (bands of 648 bp and 148 bp; Fig 1). The four parents (III:1, III:2, III:4 and III:5) showed

heterozygosity for one WT allele (648 bp and 148 bp) and on mutant allele (403 bp, 245 bp and 148 bp; Fig 1).

Discussion

HSF4 (OMIM 602438) belongs to the family of heat shock transcription factors that regulate the expression of heat shock proteins in response to different cellular stresses, such as oxidants, heavy metals, elevated temperature, bacterial and viral infections. Two isoforms, *HSF4a* and *HSF4b* resulting from two alternative splice sites for exon 8 and 9 have been reported [20,21]. *HSF4a* actively represses transcription of other heat shock factor genes by binding directly to the heat shock elements. *HSF4b*, which has 30 additional amino acids, acts as an activator of transcription. It has been demonstrated that the additional 30 amino acids are responsible for this activity [22,23].

Here, we report the first nonsense mutation in exon 11 of a large Afghani/Pakistani family. DNA sequencing revealed the transition c.1213C>T (p.Arg405X). This mutation is predicted to cause a premature termination, presumably resulting in a complete loss of function of the aberrant *HSF4* protein in affected homozygotes. This causes a severe phenotype with autosomal recessive congenital cataracts. Indeed, the *HSF4* gene that has been reported responsible both for autosomal dominant and autosomal recessive cataracts. The association of the *HSF4* gene with two different modes of Mendelian inheritance for congenital cataracts can be explained by the location and severity of the mutations in both cases. Interestingly, all the known dominant mutations in *HSF4* lie within the α -helical DNA binding domain, whereas the recessive mutations lie outside this highly conserved functional domain. To-date, only eight different mutations in *HSF4*

have been reported, three of them are causing autosomal dominant cataracts, three are causing autosomal recessive cataracts, and two mutations were found in sporadic cases. In addition to this novel nonsense mutation in exon 11, the already known mutations in *HSF4* comprise six missense mutations, one deletion, and one splice site mutation (Human Mutation Gene Database, <http://www.hgmd.cf.ac.uk/>). Exon 11 is encoding the so called DHR domain of the *HSF4* protein, the importance of which was already demonstrated by identifying a 5' splice site mutation in intron 12 causing skipping of exon 12 in a large Tunisian family [24]. Two previously identified mutations (p.Arg175Pro and c.595_599delGGGcc) in Pakistani families showed recessive cataracts and are located in the HR-A and HR-B domains of the *HSF4* protein again demonstrating the extreme genetic heterogeneity of the Pakistani population [25]. Historically, Pakistan has experienced not only internal migrations across the Indus valley but also successive waves of migrations from northwest. In more recent times, the region experienced massive migrations of refugees from Afghanistan. So, the Pakistani society is composed of many ethnic groups and many of these are common to populations of India, Afghanistan, Bangladesh and Iran. Concerning the clinical phenotype caused by mutations in this transcription factor, it is unclear, why mutations in *HSF4* that is also expressed in tissues including the heart, muscle, lung and brain should cause only nonsyndromic cataracts [3,8] and more comprehensive studies will be needed to answer this question.

Conclusion

As a conclusion, we have shown the first nonsense mutation in *HSF4* causing autosomal recessive cataracts in a large consanguineous Pakistani family. Identification of the

mutations causing cataracts will lead to a better understanding of the mechanism involved in vision loss and will help to understand the functional and structural diversity of cataract genes and the variety of proteins they encode.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

NS performed the clinical ascertainment and linkage analysis, IG performed the mutation screening, NK helped in linkage analysis and clinical evaluation, AMC provided funds for this study, CK analyzed and supervised the mutation identification, JA designed, and supervised the study, CK and JA drafted the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1: Pedigree and restriction enzyme digestion analysis of family BUIT-CA01 with 100 bp ladder (L) and control (C): Restriction enzyme (*HphI*) digest demonstrates the wild type allele (648 bp and 148 bp) in individuals II:4 and III:3 and homozygous mutant allele (403 bp, 248 bp and 148 bp) in affected individuals IV:1, IV:2, IV:3, IV:4, IV:5, IV:6, IV:7 and IV:8 while the parents III:1, III:2, III:4 and III:5 carry one WT and one mutant allele.

Figure 2: Results of sequence analysis of exon 11 of the *HSF4* gene: A homozygous C>T transition was found at nucleotide 1213 in the affected individual (IV:4), predicted to change the amino acid arginine into a stop codon, while an unaffected family member (III:3) was homozygous for the WT-allele.

BUIT-CA01

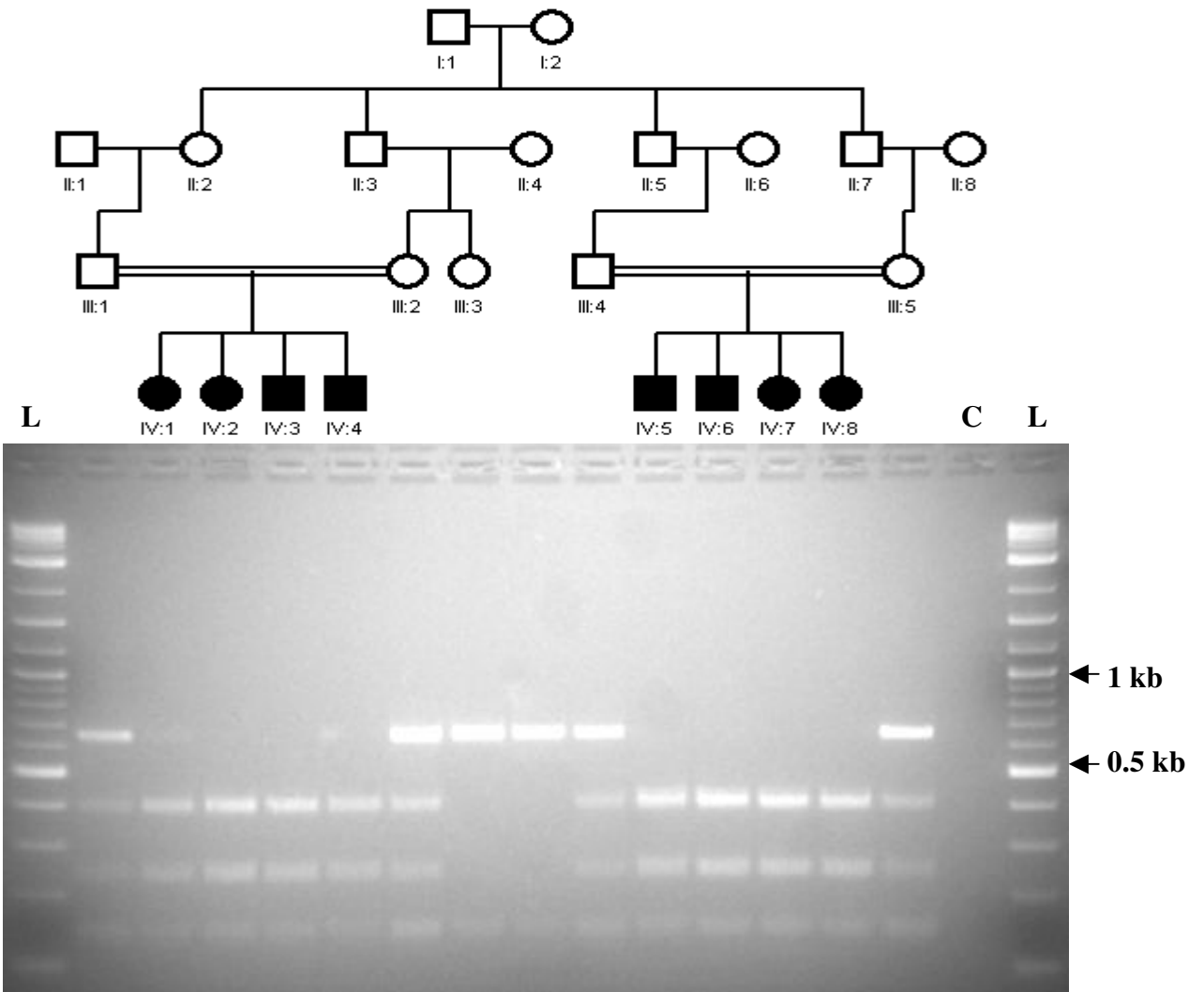


Figure 1

HSF4_c.1213C>T (p.R405X)

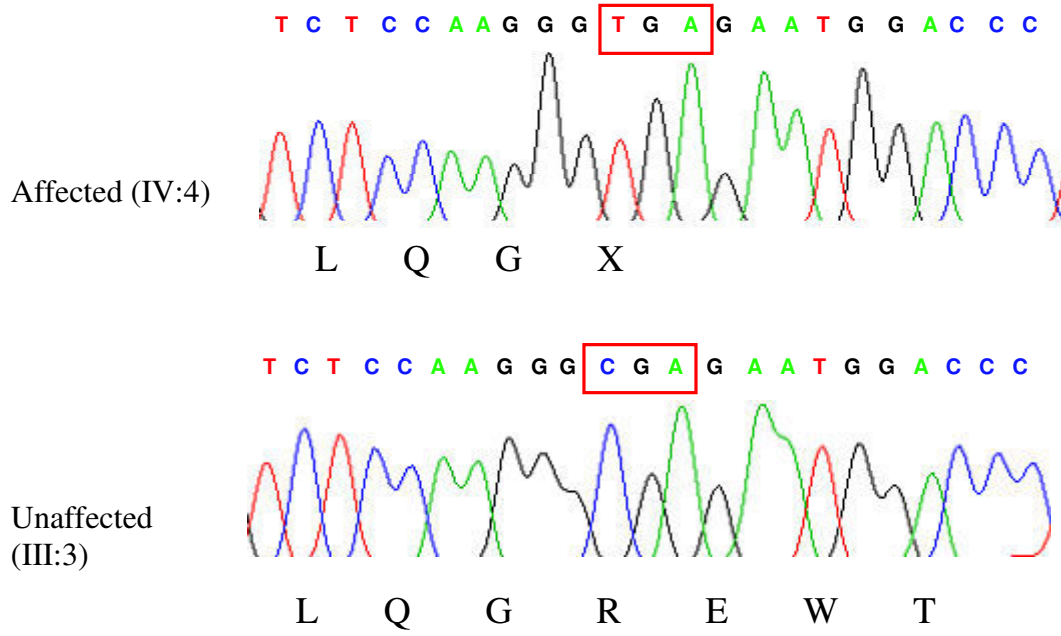


Figure 2