SHORT COMMUNICATION

A novel homozygous mutation in *HSF4* causing autosomal recessive congenital cataract

Mahdiyeh Behnam^{1,5}, Eri Imagawa^{2,5}, Ahmad Reza Salehi Chaleshtori³, Firooze Ronasian¹, Mansoor Salehi⁴, Noriko Miyake² and Naomichi Matsumoto²

Cataract is defined as opacity in the crystalline lens and congenital cataract occurs during the first year of life. Until now, mutations of more than 50 genes in congenital cataract have been reported with various modes of inheritance. Among them, *HSF4* mutations have been reported in autosomal dominant, autosomal recessive and age-related forms of cataract. The inheritance patterns of these mutations depend on their mutational positions in *HSF4*: autosomal dominant or recessive mutations are respectively found either in a DNA-binding domain or in (or downstream of) hydrophobic repeats. Here we report a novel homozygous *HSF4* mutation (c.521T>C, p.Leu174Pro) in two affected sibs of an Iranian consanguineous family using whole exome sequencing. The mutation is predicted as highly pathogenic by *in silico* analysis (SIFT, Polyphen2 and MutationTaster) and is not found in any of control databases. This mutation is located in a hydrophobic repeat of the *HSF4* protein, which is consistent with the mode of inheritance as an autosomal recessive trait.

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INTRODUCTION

Cataract is a common ocular disorder with lens opacities or cloudiness and may be classified, based on the onset ages,¹ as congenital (occurring within 1 year after birth), juvenile (occurring from 1 to 10 years of age), presenile (before 45 years) or as age-related (after 45 years) forms. In particular, congenital cataract (CC) is seen in 3–6 of 10 000 live births, and patients might have irreversible amblyopia and blindness without any early treatments.² It is known that ~8–25% of CC cases are hereditary such as autosomal dominant, autosomal recessive and X-linked recessive modes of inheritance.^{2,3} To date, mutations of at least 86 genes have been reported in cataract (Human Gene Mutation Database, Professional release 2015.1, <http://www.hgmd.org>), and among them, mutations of more than 50 genes cause CC.^{1,4,5}

HSF4 mutations have been found in CC both with autosomal dominant and recessive modes of inheritance.^{6–12} Interestingly, all dominant mutations reported are located in a DNA-binding domain close to the N-terminal region of *HSF4*, whereas the autosomal recessive mutations reside within hydrophobic repeats (HR-A/B) or downstream of the hydrophobic repeat.^{6–12} Here we report a novel homozygous mutation in *HSF4* with two affected patients showing autosomal recessive CC in an Iranian family. This is the second report of a missense mutation leading to autosomal recessive CC.

MATERIALS AND METHODS

Subjects

An Iranian family comprising of the parents, two sons and a daughter was investigated in this study. The brothers (VI-1 and VI-2 in Figure 1a) were affected with CC and nystagmus. Informed consent was obtained from the parents and blood samples were collected from the familial members at the Medical Genetics Center of Genome (Isfahan, Iran). DNA was extracted from peripheral blood leukocytes using QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Institutional Review Boards of Yokohama City University School of Medicine approved this study.

Candidate gene identification

Whole exome sequencing was performed in one affected individual (VI-2 in Figure 1a) as described in Supplementary Methods. On the basis of the pedigree tree, we identified the autosomal recessive or X-linked recessive variants in 86 known genes, which are known to be mutated in cataract (Supplementary Table 1) and then validated the candidate variants by the Sanger sequencing method. PCR products amplified with genomic DNA as a template were sequenced on an ABI3500xL sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA).

RESULTS AND DISCUSSION

Regarding whole exome sequencing, 92.5% of RefSeq coding sequences were covered by 20 or more reads in the patient, and six candidate variants in 86 known genes were identified (Table 1).

¹Medical Genetics Center of Genome, Isfahan, Iran; ²Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; ³Department of Medical Genetics, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran and ⁴Division of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

⁵These authors contributed equally to this work.

Correspondence: Dr N Miyake or Dr N Matsumoto, Department of Human Genetics, Yokohama City University Graduate School of Medicine, Fukuura 3-9, Kanazawa-ku, Yokohama 2360004, Japan.

E-mail: nmiyake@yokohama-cu.ac.jp or naomat@yokohama-cu.ac.jp

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Figure 1 Genetic analysis of the family with congenital cataract. (a) Family pedigree with the mutation. (b) Electropherograms of affected individuals (VI-1 and VI-2) and unaffected family members (V-3, V-4 and VI-3). The *HSF4* homozygous mutation (c.521T>C, p.Leu174Pro) was found in VI-1 and VI-2 (arrows). (c) Evolutionary conservation of p.Leu174 in *HSF4*. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Summary of 6 candidate variants in 86 known genes

Gene	Accession number	Nucleotide change	AA change	SIFT	Polyphen2	MutationTaster	MAR ^a (VI-2)	In-house exome ^b
<i>PXDN</i>	NM_012293.2	c.3850G > T	p.Val1284Leu	0.07	0.162	Disease causing	0.41	0/575
<i>GCNT2</i>	NM_145655.3	c.816C > G	p.Asp272Glu	1.00	0.001	Polymorphism	1.00	569/575
<i>HSF4</i>	NM_001040667.2	c.521T > C	p. Leu174Pro	0.00	0.999	Disease causing	1.00	0/575
<i>MED13</i>	NM_005121.2	c.5025G > T	p.Gln1675His	0.14	0.001	Disease causing	0.37	0/575
<i>ADAM9</i>	NM_003816.2	c.226G > A	p.Glu76Lys	0.97	0.097	Polymorphism	0.53	0/575
<i>TRPM3</i>	NM_001007471.2	c.677-2->T	—	ND	ND	ND	1.00	257/575

Abbreviations: AA, amino acid; ND, no data.

^aMAR, mutation allele rate (fraction of the sequence reads with the mutation among total reads).

^bNormal Japanese controls ($n=575$) boxes, respectively.

Figure 2 Pathogenic *HSF4* mutations in human *HSF4* protein. The previously reported dominant and recessive mutations were shown above and below the *HSF4* protein, respectively. The novel p.Leu174Pro was found in the hydrophobic repeats (HR-A/B) region (yellow box). The DNA-binding domain (DBD) and the downstream of hydrophobic repeat (DHR) are indicated as blue and green. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Based on the hypothesis that this condition is caused by an autosomal recessive or X-linked recessive inheritance, only homozygous or compound heterozygous mutations in autosomes and hemizygous mutations in the X chromosome were analyzed. Only three homozygous variants remained: *GCNT2* (NM_145655.3, c.816C>G), *TRPM3* (NM_001007471.2, c.677-2->T) and *HSF4*

(NM_001040667.2, c.521T>C, p.Leu174Pro). *GCNT2* and *TRPM3* variants were unlikely to be pathogenic as they were observed in 569 and 257 of 575 individuals, respectively, in our in-house exome database. Therefore, only one homozygous missense mutation in *HSF4* remained as a candidate. In contrast, this variant was not found in the following variant databases: our in-house Japanese exome

database ($n=575$), 1000 Genomes database, Exome Aggregation Consortium browser (July, 2015 accessed), or NHLBI Exome Sequencing Project (ESP6500). The Sanger sequencing revealed that the mutation co-segregated with CC affection status in the pedigree (Figure 1b). The *HSF4* variant was predicted to be pathogenic based on the web-based programs (SIFT = 0, Polyphen2 = 0.999, Mutation-Taster = disease causing) and the altered amino acid was highly evolutionally conserved (from *Danio rerio* to *Human sapiens*) (Figure 1c). The substitution of p.Leu174Pro is located within the hydrophobic repeats (HR-A/B) (Figure 2).¹³

The *HSF4* gene at 16q22.1 encodes a heat-shock transcription factor that forms the homotrimer repressing and/or activating downstream target genes such as *FGF1*, *FGF4*, *FGF7* and *CRYGC* required in lens development.¹⁴ In *HSF4* protein, three functional regions were recognized. The DNA-binding domain has a role for the DNA-binding mediated heat-shock element (Figure 2).^{6,9,11,12} Missense mutations found in DNA-binding domain may have a dominant negative effect obstructing both normal and mutant *HSF4* proteins.⁷ The HR-A/B and the downstream of hydrophobic repeat are essential for a trimeric formation and transcriptional activation of *HSF4*, respectively.^{13,15} The novel mutation (p.Leu174Pro) identified in HR-A/B was also found in autosomal recessive CC, supporting the previous finding.⁷

All recessive *HSF4* mutations previously reported showed significant reductions of the transcriptional activity by luciferase assay, suggesting that they were loss-of-function mutations.^{13,16} Some dominant missense mutations in *HSF4* (p.Ala19Asp, p.Arg73His, and p.Leu114Pro) have also shown a decreased transcriptional activity resembling recessive mutations.¹⁶ Thus, the CC-associated *HSF4* mutations could be loss-of-function regardless of their inheritance pattern, including p.Leu174Pro. It was previously reported that *Hsf4*-null mice presented cataract with abnormal lens fiber cells, supporting that recessive (loss-of-function) *HSF4* mutations lead to CC in humans.¹⁴ Therefore, the p.Leu174Pro in *HSF4* is highly likely causative for CC in this family, though we did not provide any functional data.

In summary, we described here one consanguineous Iranian family showing CC caused by a novel *HSF4* mutation. More mutations should be accumulated to thoroughly differentiate recessive and dominant mutations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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