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SHORT COMMUNICATION

Identification of a novel *GPR143* mutation in a large Chinese family with congenital nystagmus as the most prominent and consistent manifestation

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Abstract Congenital nystagmus is characterized by involuntary, rhythmical, repeated oscillations of one or both eyes. We studied a large Chinese family with nystagmus as a prominent and consistent manifestation phenotype in nine patients to map and identify a disease-causing gene for nystagmus. X-linked recessive inheritance was observed in the family, and foveal hypoplasia was detected in some of the nine patients. The disease gene was mapped to an approximately 10.6 Mb region flanked by *DXS996* and *DXS7593* on Xp22 with a significant peak multipoint LOD score. Analysis of 21 candidate genes in the region revealed a novel p.S89F mutation in the second transmembrane domain of GPR143, a G protein-coupled receptor which causes ocular albinism when mutated. All male patients in the family were hemizygous for the mutation; the female carriers were heterozygous for the mutation. The p.S89F mutation was not identified in 100 normal females or 100 normal males. Our results indicate that a mutation in the *GPR143* gene can cause a variant form of ocular albinism, with congenital nystagmus as the most prominent and only consistent finding in all patients in this Chinese family. These results expand the spectrum of clinical phenotypes associated with *GPR143* mutations.

Keywords Congenital nystagmus and consistent manifestation · *GPR143* · Mild ocular albinism · Linkage analysis · Mutation

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Introduction

Congenital nystagmus (CN) is a common oculomotor disorder with a frequency of 1/1,500 live births (Patton et al. 1993). It is characterized by bilateral uncontrollable ocular oscillations, reduced vision, and onset typically at birth or within the first few months of life. Patients' oscillations can be horizontal, vertical, or torsional, or any combination of these, although horizontal are the most common (Patton et al. 1993; Preising et al. 2001; Zhang et al. 2005). Reduced vision and poor depth perception are the major symptoms of patients with nystagmus. The etiology or molecular pathogenic mechanism of CN is largely unknown.

Multiple inheritance patterns have been described for CN, including X-linked dominant and X-linked recessive

(MIM 310700), autosomal dominant (MIM 164100), and autosomal recessive (MIM 257400) forms (Kerrison et al. 1999). Among these types, X-linked inheritance is the most common type, and two genetic loci for X-linked CN have been mapped to Xp11.3–11.4 and Xq26–q27, respectively (Cabot et al. 1999; Guo et al. 2006; Kerrison et al. 1999; Zhang et al. 2005). Xq26–27, also known as *NYS1*, is the most common locus; the responsible gene has recently been identified as a FERM domain-containing 7 gene (*FRMD7*) (Tarpey et al. 2006). Although three genetic loci for the autosomal dominant form have been mapped to chromosomes 6p12, 7p11.2, and 13q31–q33 (Klein et al. 1998; Kerrison et al. 1996, 1998; Ragge et al. 2003), the specific genes at these genetic loci have not yet been identified.

Nystagmus can also occur in the setting of other retinal diseases, including ocular albinism resulting from mutations in the *GPR143* gene, also known as *OA1*, encoding a G-protein-coupled receptor, and X-linked congenital stationary night blindness, caused by mutations in a calcium channel gene *CACNA1F* (Gottlob 2001).

We have identified and characterized a large six-generation Chinese family with X-linked recessive nystagmus. Nine individuals in the family are affected, all males. In addition to nystagmus some patients also suffer from foveal hypoplasia, myopia, amblyopia, and astigmatism. Linkage analysis of the family mapped the disease-causing gene to Xp22.11–Xp22.32 with a significant multipoint LOD score of 8.89. Mutation analysis of candidate genes in the region suggested that a novel mutation in the *GPR143* gene causes the disease in the family.

Subjects and methods

Study subjects and isolation of human genomic DNA

The study participants were identified and enrolled at the People's Hospital of Nanyang County, Henan province, P.R. China. Informed consent was obtained from the participants in accordance with study protocols approved by the Ethics Committee of Huazhong University of Science and Technology. This study adhered to the tenets of the Declaration of Helsinki.

The participants were clinically examined at the People's Hospital of Nanyang County, the Eye Center of Peking University, and the Union Hospital of Huazhong University of Science and Technology. Ocular examinations were performed by slit lamp biomicroscopy and direct and indirect ophthalmoscopy.

DNA was extracted from peripheral whole blood by use of a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA).

Linkage and candidate genes mutation analysis

Linkage analysis of the genotyping data was performed as described elsewhere (Wang et al. 2005). Genotyping was performed using 33 polymorphic markers covering the entire X chromosome. Eighteen markers that span the X-chromosome by every 10 cM were from the ABI Prism Linkage Mapping Set MD10 (performed as instructed by the manufacturer). The other markers were identified from the Marshfield Medical Genetics database (http://research.marshfieldclinic.org/genetics/) and used for fine mapping. The disease was specified as an X-linked recessive trait with penetration of 99% in males. We assumed a gene frequency of 0.00001. Pairwise logarithms of the odds (LOD) scores and multi-point LOD scores were calculated with the Linkage Package 5.2 software.

Mutation analysis was conducted for all exons and exon–intron boundaries of candidate genes using direct DNA sequence analysis (Wang et al. 2005).

Restriction fragment length polymorphism (RFLP) analysis

The wild type allele contains a *Bsl*I restriction site, and mutation 266C → T (GenBank accession number: Z48804) disrupts this site. This enabled use of RFLP analysis to confirm the mutation and to test whether the mutation co-segregated with the disease in the family. Exon 2 of *GPR143* containing the mutation was PCR-amplified for all members of the family and for 200 (100 males and 100 females) unrelated healthy Chinese individuals, as described above. The 237-bp PCR product was digested with 1 unit *Bsl*I (NEB, USA) at 37°C overnight, and the resulting digestion products were separated on 2.5% agarose gel. The wild-type product can be cut by *Bsl*I and yielded two bands of 144 and 93-bp. The mutant product cannot be cut by *Bsl*I, and only one 237-bp band was observed after electrophoresis.

Results

Clinical characterization of patients

We identified a large, six-generation, non-consanguineous Chinese family with 36 living members, among whom were eight living patients with nystagmus (Fig. 1A). Investigation of family history revealed one deceased member had also had nystagmus. All affected individuals were males, and the inheritance pattern of the disease in the family was typical X-linked recessive (Fig. 1A).

The proband (Fig. 1A, V-10) developed nystagmus with the horizontal oscillation before the age of six months. The

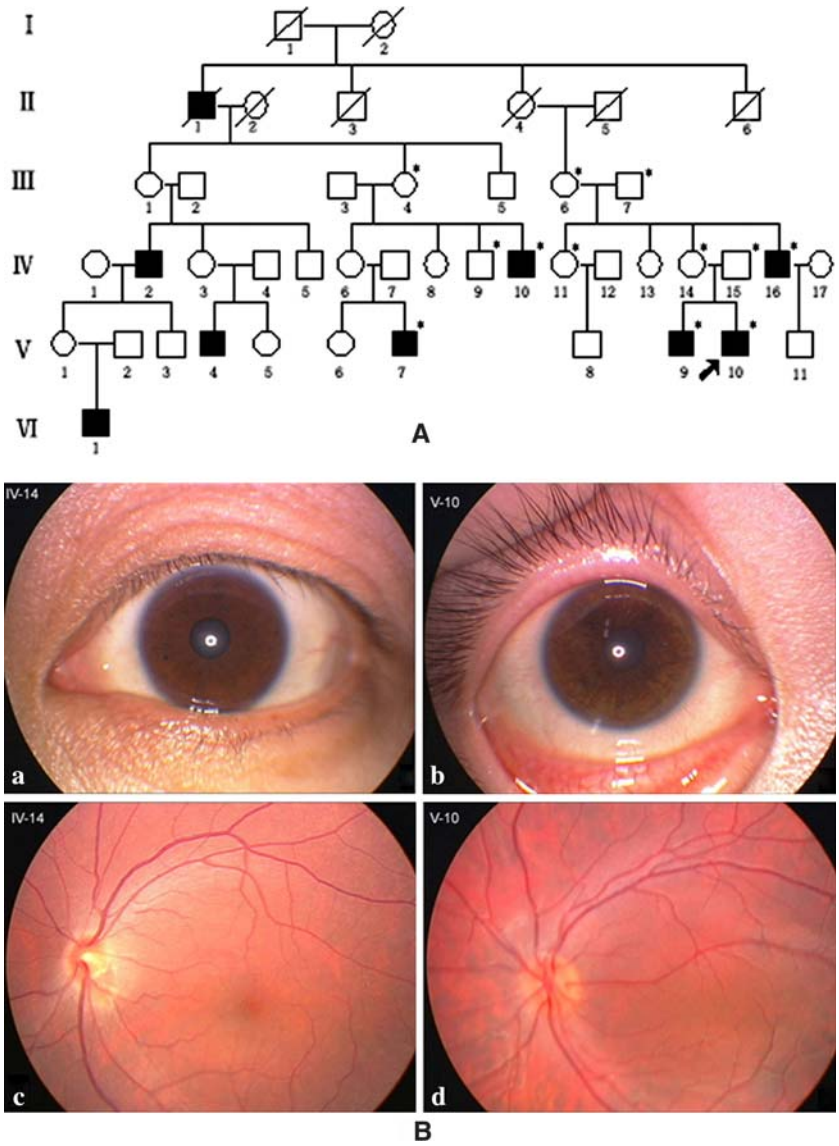

Fig. 1 **A** The pedigree structure of a Chinese family with CN. The proband is marked with an *arrow*. The family members participating in both clinical and follow-up mutation analysis are indicated by *asterisks*. **B** Iris and fundus photos from mutation carrier IV-14 (*a*, *c*) and the proband V-10 (*b*, *d*)

patient later developed astigmatism and amblyopia. The visual acuity of right and left eyes was 20/60 and 20/50, respectively. Examination of the iris revealed clear veins and normal response to the light (Fig. 1B, b). Fundus examinations revealed the clear-cut boundary of optic disc, normal distribution of blood vessels, and retina pigmentation (Fig. 1B, d). Foveal dysplasia was identified in the proband (Fig. 1B, d) and three other patients (IV-10, IV-16, V-9) (data not shown). In the skin and hair of these patients pigmentation was normal.

Patients IV-10, IV-16, V-7, and V-9 and obligate female carrier IV-14 were also characterized clinically (Fig. 1; Table 1). Pigmentation of skin and hair was normal. The pigments in the macula were clear and regular in patients IV-10 and carrier IV-14, but not in patients V-9 and IV-16. The myopia arcs were present in patients V-9, IV-16, and carrier IV-14 (Fig. 1B, c; Table 1). The female carriers in this family had normal visual acuity, except for carrier IV-14, and were free from nystagmus.

Genetic linkage analysis

Analysis of the pairwise LOD scores identified positive linkage to marker *DXS987* with a peak LOD score of 1.81 at a recombination fraction of 0 (data not shown). Additional markers flanking *DXS987* were genotyped in the family and the genotyping data were used for multipoint LOD score linkage analysis. Multipoint LOD scores reached a significant level and a peak multipoint LOD score of 8.89 was obtained at *DXS987* (data not shown). Our results suggest that the clinical phenotype in the family under study is linked to *DXS987* on Xp22. The result of

Table 1 Clinical characteristics of five affected males (IV-10, IV-16, V-7, V-9, V-10) and one carrier (IV-14) in a Chinese CN family with *GPR143* mutation p.S89F

ID#	Age	CN	Iris (a)	Fundus phenotype					VA (R/L)	Astigmatism
				b	c	d	e	f		
IV-10	26	+	+	+	–	+	+	–	20/40 20/60	+
IV-16	31	+	+	+	+	–	–	+	20/650 20/200	+
V-7	3	+	+	+	–	+	–	–	20/40 20/50	+
V-9	18	+	+	+	+	–	–	+	20/200 20/250	+
V-10*	18	+	+	+	+	+	–	–	20/60 20/50	+
IV-14	40	–	+	+	+	+	+	+	20/200 20/200	–

ID# is the patient identification number in Fig. 1A. Age is the age of diagnosis in years. CN, congenital nystagmus; VA, visual acuity. *Patient V-10 had amblyopia

a Normal iris, normal response to light and normal pigmentation (+ yes; – no)

b Clear boundary of disc (+ yes; – no)

c Mottling retina pigmentation (+ yes; – no)

d Clear and neat pigmentation in macula (+ yes; – no)

e Existence of reflex light in fovea centralis (+ yes; – no)

f Myopia arc (+ yes; – no)

haplotype analysis defined the boundaries of the disease locus between *DXS996* and *DXS7593*, a region of approximately 10.6 Mb.

Identification of a novel mutation in *GPR143*

Twenty-one genes located in the disease locus were analyzed as the candidate genes for nystagmus on the basis of their potential physiological functions and their expression in the nervous system. Direct sequence analysis revealed mutation of a C to T transition at nucleotide 266 of *GPR143* (or OA1; GenBank accession # Z48804) in the proband and his mother (carrier) (Fig. 2a). The mutation leads to a substitution of a highly conserved amino acid residue serine with a phenylalanine residue at codon 89 (p.S89F). Detection of mutation p.S89F was further confirmed by RFLP analysis which showed the presence of the hemizygous mutant allele (237-bp band) in male patients IV-2, V-1, V-2, and IV-6 (Fig. 2b), both wild type and mutant alleles (143 and 94 bps bands) in female carriers (individuals III-1, III-2, IV-4 in Fig. 2b), and homozygous and hemizygous wild type alleles in normal family members (individuals IV-1, III-3, IV-3, IV-5 in Fig. 2b). Patient V-7 in Fig. 1 also carried the mutation (data not shown). RFLP analysis also showed that the p.S89F mutation was not present in 200 normal controls (100 males and 100 females). The p.S89F mutation is located in the critical transmembrane segment II of the G protein-coupled receptor (Fig. 2c). The p.S89F mutation occurs at a residue that is evolutionarily highly conserved from *Xenopus* and fish to humans (Fig. 2d). These results strongly suggest that the p.S89F change is a pathogenic mutation for the disease in the family.

Discussion

We have identified and characterized a six-generation Chinese family with X-linked recessive nystagmus in all patients and foveal hypoplasia in some patients (Fig. 1A). Linkage analysis with markers covering the entire X chromosome and follow-up haplotype analysis mapped the disease gene on chromosome Xp22. Mutation analysis identified a novel p.S89F mutation in the *GPR143* gene. No mutation was identified in twenty other candidate genes analyzed. These results strongly suggest that the p.S89F mutation causes the disease in the family.

The *GPR143* gene (or *OA*1) consists of nine exons and encodes a 439-kDa protein of 404 amino acids with homology to seven transmembrane segments, a G protein-coupled receptor (Bassi et al. 1995). It was originally identified by positional cloning as a gene that, when mutated, caused ocular albinism, a disease characterized by absent or decreased ocular pigmentation, reduced visual acuity, and photophobia (Schiaffino et al. 1996). Although many mutations in *GPR143* have been identified in patients (Camand et al. 2003; Schiaffino and Tacchetti 2005; Mayeur et al. 2006), the p.S89F mutation is the first *GPR143* mutation found in the Chinese population.

The achiasma syndrome has also been observed in a patient with nystagmus (Korff et al. 2003). Misrouting of the optic fibers in some patients with ocular albinism has been revealed by use of visually evoked magnetic fields (VEFs) (Ohde et al. 2004; Lauronen et al. 2005). Functional magnetic resonance imaging (fMRI) studies of some patients with oculocutaneous albinism revealed abnormalities in the optic nerve head (Kasmann-Kellner et al. 2003; Morland et al. 2001). We performed fMRI on patient V-10 but no remarkable abnormalities were identified.

Nystagmus has been reported in ocular albinism patients with mutations in *GPR143* (Preising et al. 2001). Genetic and clinical analysis revealed that the Chinese family under study has a variant phenotype of ocular albinism, because a mutation in *GPR143/OA1* was identified, and foveal hypoplasia and reduced visual acuity, in addition to nystagmus, were observed for some patients in the family.

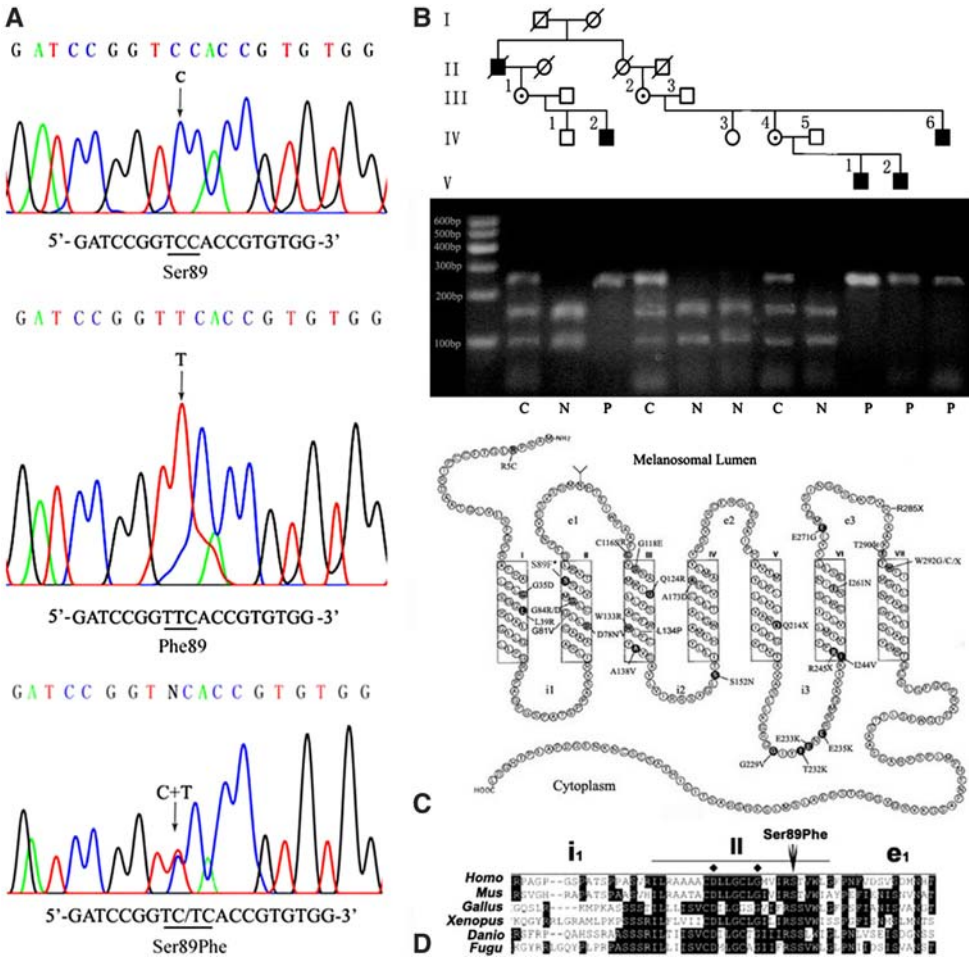

Fig. 2 **a** Identification of a novel mutation c.266C > T (p.S89F) in the *GPR143* gene in the Chinese family with CN. DNA sequences for a normal family member (IV-9 in Fig. 1) (*upper*), the proband V-10 (*middle*), and female carrier IV-14 (*bottom*) are shown. The C to T change at codon 89 results in substitution of a Ser residue by a Phe residue in exon 2 of the *GPR143* gene (GenBank accession number Z48804). **b** RFLP analysis showing that *GPR143* mutation p.S89F co-segregates with nystagmus in the family. The first lane shows 100 bp molecular weight ladder. The p.S89F mutation abolishes a *BSI*I restriction site. The PCR fragment was 237-bp. The wild type PCR product can be cut by *BSI*I, yielding two shorter DNA fragments of 143 and 94 bp. The fragment containing mutation p.S89F cannot be cut by the *BSI*I enzyme. Separate RFLP analysis showed that individual V-7 in Fig. 1A also carried the mutation (data not shown). The PCR primers used for RFLP analysis are 237-1/forward, 5′-TTTCCAAAGCAAGAAGTCAGC-3′ and 237-1/reverse, 5′-TGCGATTTGAGGAGCATAAG -3′. **c** The putative structure of the GPR143 receptor with seven putative transmembrane α-helices (TM I to VII) (modified from d'Addio et al. 2000). The GPR143 protein is inserted within the melanosomal membrane with the N-terminus toward the melanosomal lumen and the C-terminus toward the cytoplasm. Hydrophilic lumenal and intracellular loops are indicated by e_{1-3} and i_{1-3}, respectively. The location of 24 independent missense mutations and three nonsense mutations (with X) identified in *GPR143* are marked (from the OA1 mutation database Camand et al. 2003; Mayeur et al. 2006). The p.S89F mutation identified in this study is indicated by an *asterisks* and is at the second putative transmembrane α-helix. **d** The alignment of amino acids around S89 revealed evolutionary conservation of this residue from *Fugu*, *Danio*, *Xenopus*, *Gallus*, and *Mus* to *Homo*. *Dark shading* indicates residues conserved in six sequences; *diamonds* indicate sites of missense mutations reported and identified in ocular alinism patients (Schiaffino et al. 2005). The location of the p.S89F mutation identified in this study is marked with an *arrowhead*

None of the patients in the family had the complete classical phenotype of ocular albinism.

GPR143 is expressed mainly in pigment cells of the skin and eyes. It is located on the membrane of an intracellular organelle—the melanosomes in pigment cells—and regulates the biogenesis and maturing of melanosomes (Preising et al. 2001; Schiaffino and Tacchetti 2005). *GPR143* mutations that cause ocular albinism are loss-of-function mutations, because many are deletion, frameshift, and nonsense mutations (Mayeur et al. 2006). Some *GPR143* mutations had defective glycosylation and were retained in the endoplasmic reticulum (Shen and Orlow 2001). A similar phenotype to human ocular albinism patients, including hypopigmentation of the ocular fundus and the presence of giant melanosomes formed by abnormal growth of single melanosomes, has also been observed in

knockout mice deficient in *GPR143* (Schiaffino and Tacchetti 2005).

The molecular pathogenic mechanism for nystagmus is unknown. It may result from instability of the ocular motor system (Dell'Osso and Flynn 1974). Changes in the ultrastructure of the extraocular muscle of congenital nystagmus patients have been detected by transmission electron microscopy (Peng et al. 1998). It is likely that the *GPR143* mutations may disrupt a key signal-transduction pathway and cause instability in the motor system controlling the eye movement. The specific molecular mechanism by which the GPR143 p.S89F mutation causes nystagmus and foveal dysplasia is not clear, however, and future functional studies may shed light on this issue.

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