Original Article

A previously unidentified deletion in G protein-coupled receptor 143 causing X-linked congenital nystagmus in a Chinese family

Jing Liu, Yanlei Jia[1], Lejin Wang, Juan Bu

Background: Congenital nystagmus (CN) is characterized by conjugated, spontaneous, and involuntary ocular oscillations. It is an inherited disease and the most common inheritance pattern is X-linked CN. In this study, our aim is to identify the disease-causing mutation in a large sixth-generation Chinese family with X-linked CN. **Methods:** It has been reported that mutations in four-point-one, ezrin, radixin, moesin domain-containing 7 gene (FRMD7) and G protein-coupled receptor 143 gene (GPR143) account for the majority patients of X-linked nystagmus. We collected 8 ml blood samples from members of a large sixth-generation pedigree with X-linked CN and 100 normal controls. FRMD7 and GPR143 were scanned by polymerase chain reaction (PCR)-based DNA sequencing assays, and multiplex PCR assays were applied to detect deletions. **Results:** We identified a previously unreported deletion covering 7 exons in GPR143 in a Chinese family. The heterozygous deletion from exon 3 to exon 9 of GPR143 was detected in all affected males in the family, while it was not detected in other unaffected relatives or 100 normal controls. **Conclusions:** This is the first report of molecular characterization in GPR143 gene in the CN family. Our results expand the spectrum of GPR143 mutations causing CN and further confirm the role of GPR143 in the pathogenesis of CN.

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Congenital nystagmus (CN) is characterized by conjugated, spontaneous, and involuntary ocular oscillations. It is an inherited disease and the most common inheritance pattern is X-linked CN. Four-point-one, ezrin, radixin, moesin domain-containing 7 (*FRMD7*) and G protein-coupled receptor 143 *(GPR143)* have been identified as the disease-causing genes for X-linked CN.[1,2] The *FRMD7* gene comprises 12 exons and encodes a protein with 714 amino acids. The *GPR143* gene consists of 9 exons and encodes a protein with 404 amino acids.

Numerous mutations in *FRMD7* gene have been reported since it was first identified in 2006.[3-8] Mutations in *FRMD7* gene are the major causes of Chinese familial X-linked CN and account for approximately 47% of Chinese patients with the disorder.[9]

GPR143 gene is also known as the ocular albinism type 1 (*OA1*) gene, as mutations in *GPR143* also cause OA1.[10] Most patients with OA1 show nystagmus and poor visual acuity. Several reports confirmed the pathogenicity of *GPR143* gene in CN family.[2,11-14]

In this study, we analyzed the variants in *FRMD7* and *GPR143* genes in a sixth-generation, nonconsanguineous CN Chinese family. We identified a deletion covering 3–9 exons of *GPR143* gene resulting in a truncated protein of 120 residues. Our results expand the spectrum of *GPR143* mutations causing CN. This is the first report of molecular characterization in the *GPR143* gene in the CN family.

Methods

A sixth-generation Han family from Hebei Province in China including six male patients participated in this study [Fig. 1]. The patients underwent complete physical and ophthalmic examinations. The Institutional Review Board approved the project, and investigators followed the principles of the Declaration of Helsinki. Informed consent was obtained from each person. One hundred normal male controls mainly from the north of China were also analyzed.

Blood specimens (8 ml) of each family member available were collected in ethylenediaminetetraacetic acid, and genomic DNA was extracted from peripheral blood cells according to a standard protocol (Roche Diagnostics Corporation, Indianapolis, IN, USA). In brief, all the exons and exon–intron boundaries of *FRMD7* and *GPR143* genes were amplified using the standard polymerase chain reaction (PCR) buffer system with primers listed in Tables 1 and 2. PCR reactions were performed in a 10 μL volume, containing 1.5 mM

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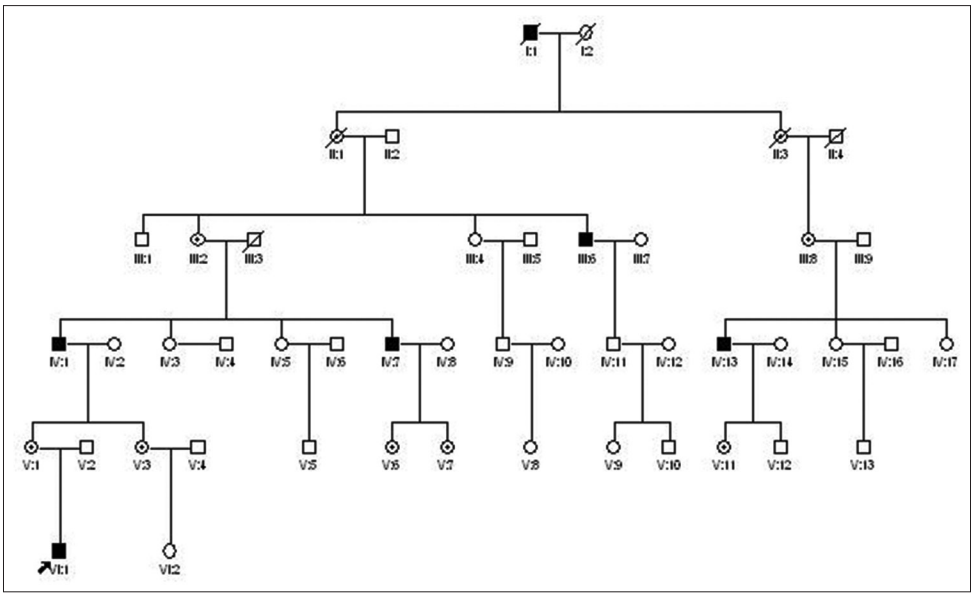

Figure 1: The pedigree of family. Squares and circles indicate males and females, the darkened symbols represent affected members, dot-marked symbols represent females who carried the mutation, and the patient above the arrow is the proband

Table 1: Primers used to amplify the exons of four-point-one, ezrin, radixin, moesin domain-containing 7

Primer name	Sequence	Melting temperature (°C)	Product size (bp)
FRMD7_E1_F	gctgagtttaagaaggctagagg	60.08	563
FRMD7_E1_R	atttgctattgttgtcccttgag		
FRMD7_E2_F	aagggtaaatttgcagatgtagc	59.64	548
FRMD7_E2_R	acaaagagggaggacaaaaactag		
FRMD7_E3_F	agggggcagattaaacgtag	59.52	505
FRMD7_E3_R	gcagtgccagaaaatgagata		
FRMD7_E4_F	gaggggacggaagaggagagc	59.10	450
FRMD7_E4_R	ggcataacccccaagtggatac		
FRMD7_E5_F	cccaaaaaggcatctgactg	58.63	375
FRMD7_E5_R	aggccatgctgtttctctctatc		
FRMD7_E6-7_F	ccaaacacacacacccctatag	58.98	851
FRMD7_E6-7_R	cctatttctgtccccatctatcc		
FRMD7_E8_F	acccctttcttgcttgcattc	59.72	440
FRMD7_E8_R	ggcaaaagaaaagacacaccatc		
FRMD7_E9_F	ggagccaagtggaaaatcagaag	59.29	480
FRMD7_E9_R	cccatcttcctccctcctagttag		
FRMD7_E10-11_F	gcgttctgagtagttgaggttgt	60.50	676
FRMD7_E10-11_R	gccagttctctccagtctataagg		
FRMD7_E12-1_F	tctggaagtaggatggcattgag	58.92	975
FRMD7_E12-1_R	tgattggctctgggacctttta		
FRMD7_E12-2_F	ccccaattagagcagaggaaagg	60.86	962
FRMD7_E12-2_R	gccaacccatactgtcaccattc		

FRMD7: Four-point-one, ezrin, radixin, moesin domain-containing 7

MgCl2, 0.4 mM of each primer, 200 μM dNTPs, 1 U Taq DNA polymerase, and 10–20 ng template DNA. Amplification was performed with an initial denaturation for 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 3 min. PCR products were purified using a PCR product purification kit (QIAquick; Qiagen, Valencia, CA, USA). The purified PCR products were sequenced using the BigDye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems, Foster City, CA, USA). Briefly, about 10 ng of template DNA was added in each reaction using a temperature program which included 25 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 15 s, and extension at 60°C for 4 min. All samples were analyzed in an

ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The *FRMD7* and *GPR143* cDNA reference sequence with GenBank accession was used (National Center for Biotechnical Information, Bethesda, Md; available at: http://www.ncbi.nlm.nih.gov).

Results

This sixth-generation pedigree showed a high possibility of an X-linked recessive inheritance pattern [Fig. 1]. All patients in this family had various reduced visual acuity with a similar pattern of nystagmus. The type of nystagmus in the family appeared to be jerk nystagmus that manifests as early as at birth. They all had reduced vision, amblyopia, and astigmatism at a different degree. There were normal pigmentation of skin and hair in all patients. Moreover, no retinal pathological changes, optic nerve lesions, any typical sign of OA1, and hypopigmentation of the iris and fundus were detected [Figs. 2-4 and Table 3].

We sequenced 12 coding exons of *FRMD7*, 9 coding exons of *GPR143*, and the adjacent intronic regions of these two genes in the patients. After a complete analysis of the coding sequence of *FRMD7* and *GPR143*, there was no mutation in *FRMD7* and no PCR products for the DNA fragments spanning exon 3 to exon 9 of *GPR143*, suggesting a large deletion in this region [Fig. 5].

The mutation was detected in all patients but not found in other unaffected members or in the 100 normal controls. It was predicted to result in a premature stop codon emerged in exon 2, resulting in a truncated protein of only 120 amino acids.

Table 2: Primers used to amplify the exons of G protein-coupled receptor 143

Primer name	Sequence	Melting temperature (°C)	Product size (bp)
GPR143_E1_F	atggcaggtttggcgctctag	61.3	766
GPR143_E1_R	gcctctcgtcctcactccatcac		
GPR143_E2_F	ctctctccctccctcctttc	61.3	361
GPR143_E2_R	ggacgtgagaacctgcattt		
GPR143_E3_F	acgtcagaggaagccagtgt	66.2	352
GPR143_E3_R	tgagctgctgtggatgtttc		
GPR143_E4_F	cctctgtgtacattttcctgacct	61.3	279
GPR143_E4_R	gctcatgtattccctgcaagac		
GPR143_E5_F	tggcttgttccagacatgag	61.3	432
GPR143_E5_R	ttctgcagctgtgttctgct		
GPR143_E6_F	cctgcttccattgccttctctg	63.9	284
GPR143_E6_R	ccctttggaacttctggtcacg		
GPR143_E7_F	ggccatgtctataccgggagtt	63.9	414
GPR143_E7_R	ccagttactcaggaggccaagac		
GPR143_E8_F	agggctctgacttctgctacgc	68	451
GPR143_E8_R	gggaggtgcaactggaagctag		
GPR143_E9_F	ccgcagtcacctatcaatcaac	63.9	583
GPR143_E9_R	ggctcttccattctcacacgt		

GPR143: G protein-coupled receptor 143

Table 3: Summary of clinical features of some affected males and carriers

Individual	Gender	Iris hypopigmentation	Albinotic fundus	Fundus hypopigmentation	Fundus foveal hypoplasia	Nystagmus
Patients						
III: 6	Male	No	No	No	No	Yes
IV: 1	Male	No	No	No	No	Yes
IV: 7	Male	No	No	No	No	Yes
IV: 13	Male	No	No	No	No	Yes
VI: 1	Male	No	No	No	No	Yes
Carriers						
III: 1	Female	No	No	No	No	No
V: 1	Female	No	No	No	No	No
V: 3	Female	No	No	No	No	No
V: 6	Female	No	No	No	No	No
V: 7	Female	No	No	No	No	No
V: 13	Female	No	No	No	No	No

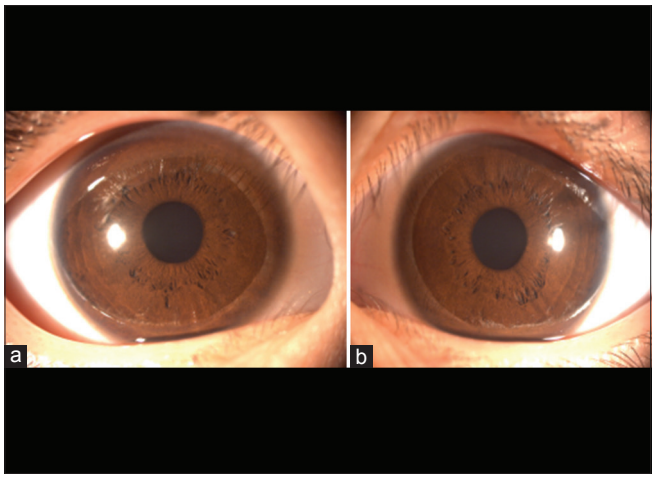

Figure 2: The iris of proband (VI1) in family, (a) right eye, (b) left eye

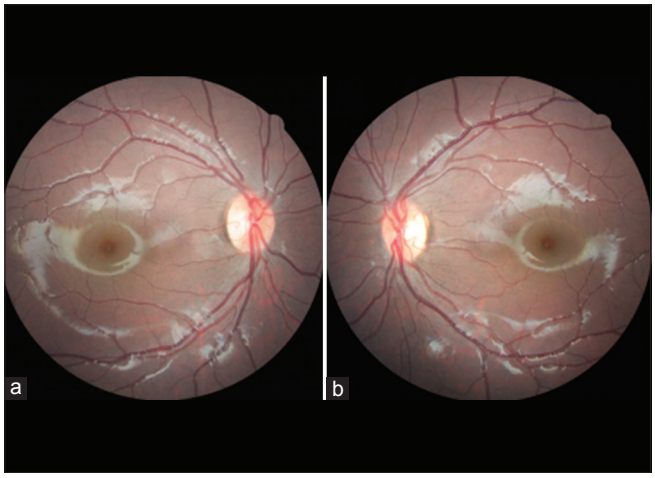

Figure 3: Photographs of fundus from proband (VI1) in family, (a) right eye, (b) left eye

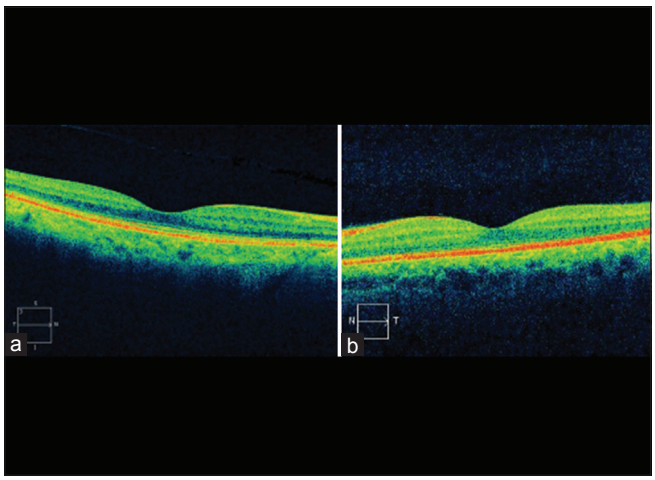

Figure 4: Photographs of OCT from proband (VI1) in family, (a) right eye, (b) left eye

Discussion

Bassi *et al*. cloned *GPR143* gene for OA1 on chromosome Xp22.3.[15] Schiaffino *et al*. detected various mutations in *GPR143* in one-third of X-linked ocular albinism.[10] Sometimes, OA1 can be ignored or misdiagnosed, the ocular disorders should be eliminated before the diagnosis of congenital motor nystagmus is made. X-linked OA1 is characterized by decreased ocular pigmentation, foveal hypoplasia, nystagmus, photodysphoria, and reduced visual acuity. All patients in this family had various reduced visual acuity with nystagmus but there were no retinal pathological changes, optic nerve lesions, or any typical sign of OA1.

We have characterized nystagmus in a sixth-generation Chinese family with a X-linked recessive inheritance pattern. No mutation was identified in the *FRMD7* gene and a novel large deletion in exon 3 to exon 9 of the *GPR143* gene. All the affected males were hemizygous for the mutation and female carriers were heterozygous for the mutation whereas other normal members of the family had no mutation. These results strongly suggested that the 3–9 exons deletion of *GPR143* gene causes the disease in the family.

Until now, more than 100 mutations of *GPR143* have been determined, including frameshift deletion and nonsense mutations. Most mutations were reported to cause ocular albinism, but some mutations were reported to cause CN without classical phenotype of ocular albinism.[12,13]

The human *GPR143* gene consists of 9 exons which encode a 439-kDa protein of 404 amino acids with homology to seven transmembrane segments, a GPR. The GPR has been shown to participate in the most common signal transduction system at the plasma membrane. Thus, it suggests that *GPR143* mediates signal transduction system and operates at the internal membranes in mammalian cells.[16] Giordano used siRNA inactivation of *GPR143* and combined morphological and biochemical methods to investigate melanosomal ultrastructure, melanosomal protein localization, and expression in human pigmented melanocytic cells. The functional loss of *GPR143* may lead to decreased pigmentation and causes formation of enlarged aberrant premelanosomes harboring disorganized fibrillar structures and displays proteins of mature melanosomes and lysosomes at their membrane.[17]

We revealed the 7-exons deletion of *GPR143* in a Chinese pedigree. The presence of the deletion in all patients and its absence in unaffected members and other 100 unrelated controls indicate that the identified deletion causes CN. The deletion in *GPR143* is predicted to result in a truncated protein of 120 amino acid residues, in which the C-terminus of GPR143 protein was deleted.

Conclusions

Our results expand the spectrum of GPR143 mutations causing CN and also confirm the role of *GPR143* in the pathogenesis of CN.

Acknowledgments

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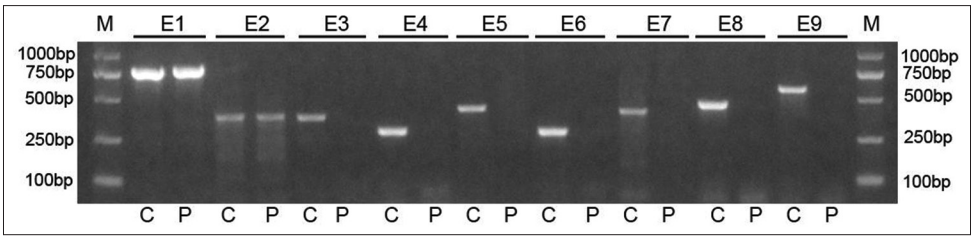

Figure 5: The polymerase chain reaction results of GPR143 gene. M: marker; E1–E9: exon1–Exon9; C: Represents carrier member; P: Represents affected member

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Conflicts of interest

There are no conflicts of interest.

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