A novel GPR143 splicing mutation in a Chinese family with X-linked congenital nystagmus

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Purpose: The purpose of the current research was to detect the underlying genetic defect in a Chinese family with X-linked congenital nystagmus and perform prenatal genetic diagnosis for their current pregnancy.

Methods: A common clinical examination and an ophthalmic evaluation were performed on the proband, one carrier, and one unaffected member. Mutation analysis of the G protein-coupled receptor 143 (GPR143) and four-point-one (4.1), ezrin, radixin, moesin (FERM) domain-containing 7 (FRMD7) genes was performed by direct sequencing of PCR-amplified exons in the proband. The detected GPR143 mutation was tested in all available family members and 200 normal controls by direct sequencing.

Results: Congenital nystagmus, obvious fundus hypopigmentation, and foveal hypoplasia were observed in the proband but not in the carriers or the unaffected members. A novel splicing mutation c.658+1 g>t not found in 200 unrelated controls was identified and co-segregated with X-linked ocular albinism (XLOA) in this family. The fetus (V:5) was hemizygous for this mutant allele.

Conclusions: We identified a novel causative mutation of GPR143 in a five-generation Chinese family with XLOA. This expanded the mutation spectrum of GPR143 and provided data elucidating the diverse and variable effects of GPR143 mutations.
The mutation of **GPR143** was tested in the 200 healthy controls by direct sequencing.

**RESULTS**

**Clinical findings:** Nine males in three successive generations of the Chinese family were affected, indicating that the disease may be inherited in an X-linked recessive pattern in this family (Figure 1). The proband presented with typical symptoms of CN, including nystagmus, amblyopia, foveal hypoplasia, and fundus hypopigmentation. Results of a computed tomography (CT) scan of the brain were normal in the proband.

- **Nystagmus and reduced visual acuity**—The nine patients all had nystagmus and poor visual acuity as a first symptom. The nystagmus was present during their first three months of life with a corrected visual acuity (VA) of 0.1–0.2. The proband’s (V:4) nystagmus was sometimes associated with head nodding, and the amplitude varied with horizontal gaze position. In all patients, the nystagmus had a tendency to diminish with age, while it rarely disappeared completely (e.g., the proband’s grandfather (III:5) continued to experience nystagmus even at age 60). The proband’s mother (IV:9), a carrier, did not experience nystagmus, and her VA was normal.

- **Fundus hypopigmentation and foveal hypoplasia**—In the present study, fundus examinations were performed in most patients, carriers, and unaffected members of the family (Table 2). Significant hypopigmentation of the ocular fundus periphery with normal pigmentation of skin and hair and foveal hypoplasia were observed in the proband (Figure 2A), while his mother (Figure 2B) and his uncle (Figure 2C) did not exhibit such symptoms.

**Mutation screening:** The nine patients and the fetus (V:5) were hemizygous for a novel splicing mutation c.658+1G>T (Figure 4A,C), while carriers were heterozygous for this genotype (Figure 4B) and unaffected members were normal (Figure 4D). The splicing mutation c.658+1G>T was not found in the 200 unrelated controls.

### Table 1. Primers and PCR conditions used to amplify genomic segments of **GPR143** and **FRMD7**.

| Primer name | Forward primer (5'-3') | Reverse primer (5'-3') | Annealing temperature (°C) | Product size (bp) |
|-------------|------------------------|------------------------|---------------------------|------------------|
| GPR143-1a   | CTCCTCCGCGCGCGAAGCATAC | CCCAGGCGACCGAAGGTC     | 66                         | 464              |
| GPR143-1b   | CCAGGCGCGCGCGAAGCATAC | ACGCAGGCGACCGAAGGTC     | 69                         | 399              |
| GPR143-2    | CAGGCGACCGCGCGAAGCATAC | GGCCAGGCGACCGAAGGTC     | 61                         | 360              |
| GPR143-3    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 59                         | 385              |
| GPR143-4    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 63                         | 334              |
| GPR143-5    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 61                         | 406              |
| GPR143-6    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 63                         | 400              |
| GPR143-7    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 63                         | 441              |
| GPR143-8a   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 63                         | 395              |
| GPR143-8b   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 63                         | 329              |
| GPR143-9    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 61                         | 330              |
| FRMD7-1     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 459              |
| FRMD7-2     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 385              |
| FRMD7-3     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 68                         | 499              |
| FRMD7-4     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 69                         | 519              |
| FRMD7-5     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 350              |
| FRMD7-6     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 353              |
| FRMD7-7     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 368              |
| FRMD7-8     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 368              |
| FRMD7-9     | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 495              |
| FRMD7-10    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 398              |
| FRMD7-11    | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 282              |
| FRMD7-12a   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 388              |
| FRMD7-12b   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 452              |
| FRMD7-12c   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 588              |
| FRMD7-12d   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 472              |
| FRMD7-12e   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 500              |
| FRMD7-12f   | CAGGCGACCGCGCGAAGCATAC | GGGCCAGGCGACCGAAGGTC     | 57                         | 500              |
of XLOA patients. To date, more than 99 different mutations of GPR143, involved in most exons, have been published [9]. Prior to 2007, XLOA in the Chinese population was infrequently reported [2], and to date, only 11 mutations of GPR143 have been described in the Chinese population including 3 missense mutations [2,7,10], 1 splicing mutation [7], 6 deletion mutations [7,11,12], and 1 duplication mutation [13]. The 11 mutations were all associated with nystagmus but without ocular albinism (OA). In the present study, the novel mutations of c.658+1G>T were found to cause CN in a large Chinese family. The cumulated number and ethnic distribution of known mutations will help further study in the pathogenesis of CN.

GPR143 on chromosome Xp22.3 contains nine exons and encodes a protein of 404 amino acids containing seven putative transmembrane domains and one potential N-glycosylation site using an asparagine at codon 106 [14]. GPR143 is mainly expressed in skin and retinal pigmented epithelial cells. The GPR143 protein is a G protein-coupled receptor (GPCR) that is embedded in the melanosome membrane [15], with the NH2-terminus of the protein in the melanosome lumen and the COOH-terminus in the cytosol.

In the present study, we found a splice site mutation of GPR143 at the exon-intron 5 boundary (c.658+1G>T) and in the 5′ consensus donor region for the splicing of intron 5–6. This mutation may lead to two possible effects: the loss of the original splicing donor or the generation of a new splice site. If the original splicing donor disappears, the exon 5 of GPR143 may be lost, thus leading to the introduction of a stop codon. This creates a truncated protein of 187 amino acids (Figure 5), which is much shorter than the normal full-length protein of 404 amino acids, and it may seriously affect the function of this protein. On the other hand, we searched for potential abnormal splice sites generated by this mutation using NNSPLICE software [16] and found some possible displaced splice sites. Being used instead of the missing original splice site, the neighboring cryptic splice sites might result in short, erratic sequences ending with a stop codon after the normal sequence. If the abnormal mRNA is actually translated and escapes degradation, the encoded protein will be truncated and dysfunctional.

XLOA, a disorder of melanosome biogenesis leading to congenital and persistent visual impairment in affected males, is characterized by CN, reduced visual acuity,

### Table 2. Summary of clinical features of some affected males and carriers.

| ID# patients | Gender | Iris hypopigmentation | Albinotic fundus | Fundus hypopigmentation | Fundus foveal hypoplasia | Nystagmus |
|--------------|--------|-----------------------|------------------|-------------------------|------------------------|-----------|
| V:4          | Male   | Mild                  | No               | Obvious                 | Obvious                | Yes       |
| III:5        | Male   | Mild                  | No               | Obvious                 | Obvious                | Yes       |
| III:7        | Male   | Mild                  | No               | Obvious                 | Obvious                | Yes       |
| III:9        | Male   | Mild                  | No               | Obvious                 | Obvious                | Yes       |
| III:17       | Male   | Obvious               | No               | Obvious                 | Obvious                | Yes       |
| IV:17        | Male   | Obvious               | No               | Normal                  | Normal                 | No        |
| IV-9         | Female | Normal                | No               | Normal                  | Normal                 | No        |
| III:12       | Female | Normal                | No               | Normal                  | Normal                 | No        |
| II:5         | Female | Normal                | No               | Normal                  | Normal                 | No        |
hypopigmentation of the iris pigment epithelium and the ocular fundus, and foveal hypoplasia [17]. XLOA is a non-progressive disorder, and visual acuity remains stable throughout life. Nystagmus has been reported in ocular albinism patients with mutations of GPR143 and is thought to be a secondary phenotype in these patients [18]. However, one of the classical OA phenotypes, ocular albinism, has rarely been observed in patients with GPR143 mutations. Fang et al. [7] found a similar splicing mutation c.658+1G>A in a family with XLOA; however, the patients’ phenotypes differed from those of our patients. While fundus hypopigmentation existed in our proband but not in theirs, both showed mild iris hypopigmentation. On the contrary, fundus hypopigmentation appeared in their carriers but not in ours. It

Figure 2. Fundi photographs. A: Fundus of the proband (V4) revealed severe fundus hypopigmentation (blue arrow) and foveal hypoplasia (white arrow). B: The fundus of the carrier mother (IV9). C: Normal fundus (IV10).
Figure 3. Iris photographs. A: Irises of the proband (V4) revealed mild hypopigmentation (blue arrow). B: Irises of the carrier mother (IV9). C: Normal irises of an unaffected member (IV10).
Figure 4. Sequencing of GPR143. A: Sequence in the proband (V4) showing a novel splicing mutation c.658+1G>T. B: Sequence in the proband's mother (IV9) revealing a heterozygous mutation. C: The sequencing result of the fetus (V5) hemizygous for the mutant allele. D: Sequence in an unaffected male member (III6) hemizygous for the wild type allele.
is still unclear why these two mutations at the same locus (c. 658+1G>T and c.658+1G>A) in GPR143 cause different phenotypes. It is possible that the new splice site generated by these two mutations will result in the expression of part intron in the 5′ consensus donor region for the splicing of intron 5–6. Thus, the mutation c.658+1G>T will introduce a new amino acid p.220Val, while c.658+1G>A will introduce p.220Asp. Different amino acids may lead to different protein structures and eventually produce different phenotypes.

The role of GPR143 in the development of the visual system is currently poorly understood [19]. In the present study, we identified a novel causative mutation of GPR143 and offered a reliable prenatal genetic diagnosis in a five-generation Chinese family with XLOA. Our findings both expand the mutation spectrum of GPR143 and provide data elucidating the diverse and variable effects of GPR143 mutations.

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