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# Molecular genetic analysis of patients with sporadic and X-Linked infantile nystagmus

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# Molecular genetic analysis of patients with sporadic and X-Linked infantile nystagmus

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**Key words**: infantile nystagmus; *FRMD7* gene; novel mutations; sporadic; X-Linked

**Word count:** 1569

## ABSTRACT

**Objectives:** Infantile nystagmus (IN) is a genetically heterogeneous condition characterized by involuntary rhythmic oscillations of the eyes accompanied with vision impairment of different severity. Two genes have been identified as major disease-causing genes for IN, *FRMD7* and *GPR143*. The aim of our study is to detect the genetic basis of both sporadic IN and X-Linked IN.

**Design:** Prospective analysis.

**Patients:** Twenty Chinese patients underwent molecular genetic analysis, including fifteen sporadic IN cases and five X-Linked IN families, was recruited in this study. We first performed PCR-based DNA direct sequencing of the entire coding region and splice junctions of the above two genes in the recruited individuals from the two pedigrees. Then mutational analysis and co-segregation confirmation were performed.

**Setting:** All clinical examinations and genetic experiments were performed in the Eye Hospital of Wenzhou Medical University.

**Results:** Two mutations in *FRMD7* gene, including one novel nonsense mutation (c.1090C>A, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in two X-Linked IN families, with the detection rate of 40% (2/5). However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases.

**Conclusions:** In conclusion, the results suggested that mutations in *FRMD7* appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

**Strengths and limitations of this study:**

- As very few studies focused on the sporadic cases, the aim of our study is to detect the genetic basis of both sporadic IN and X-Linked IN.
- Two mutations in FRMD7 gene, including one novel nonsense mutation, were identified in two X-Linked IN families.
- The results suggested that mutations in FRMD7 appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. More samples are required in the future study.

## INTRODUCTION

Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes involuntary, rapid and repetitive movements of the eyes. Signs of IN usually appear at birth or develop within the first six months of life. The incidence of all forms of infantile nystagmus is estimated to be 14 per 10,000 individuals.[1] The most common symptom of IN is visual acuity impairment, which is caused by excessive motion of images on the retina or by the movement of images away from the fovea. The abnormal eye movement sometimes become worsen when an affected person stares at an object or is feeling anxious. Patients usually tend to tilt their head in order to compensate for the abnormal eye movement.[2] Patients' eye oscillations can be horizontal, vertical, torsional, or combination of the three, and horizontal is the most common. To date, the pathogenesis of IN remains unclear. Many researchers attribute the disease to the abnormal control of the part of the brain that is in charge of ocular motor.[3] Currently, there is no effective treatment for IN, only very limited surgical,

optical or pharmaceutical therapies have been employed to improve nystagmus waveforms and vision acuity.[4,5] With the exception of idiopathic infantile nystagmus, IN can be complicated by other diseases like albinism, achromatopsia, Leber congenital amaurosis and visual deprivation at early age. It present as a component of other neurological syndromes and neurologic diseases.[6]

Infantile nystagmus can be inherited as an X-Linked, an autosomal recessive or an autosomal dominant disease with incomplete penetrance and variable expressivity. Although the inheritance pattern is heterogeneous, the most common form of IN follows that of an X-Linked pattern. To date, two major genes have been defined as the causative genes of hereditary X-Linked infantile nystagmus (XLIN).[7] The *FRMD7* gene contains an N-terminal FERM domain with high conservation and FERM-adjacent domain without significant homology. Mutations in *FRMD7* gene is currently the most common cause of XLIN. It is mainly expressed in the retina and in parts of the brain that coordinate eye movement, like the cerebellum and the lateral ventricles.[8] Although the exact function of this gene is still unclear, previous research suggests that *FRMD7* gene mutations may lead to nystagmus by disrupting the normal development of certain nerve cells in the brain and the retina. Most studies focus on its N-terminal FERM domain which may play a role between the plasma and actin cytoskeleton. Previous studies also suggest a link between membrane extension during neuronal development and remodeling of the actin cytoskeleton.[9] The other disease-causing gene of XLIN, *GPR143*, encodes a protein that binds to heterotrimeric G proteins and affects pigment production in the iris, retinal pigment epithelium and skin.[10] This protein is thought to be involved in intracellular signal transduction. Mutations in *GPR143* have been shown to cause X-Linked ocular albinism type 1(OA1), which is a multi-symptom syndrome that could lead to reduced

visual acuity, nystagmus, strabismus and so on.[11,12] However, a deletion mutation of *GPR143* gene has also been reported in a Chinese IN family without typical phenotype of ocular albinism.[13] Nearly half of X-Linked IN families have genetic defects in the *FRMD7* gene. However, very limited studies investigate the genetic basis of sporadic cases with IN.

In this study, we recruited a Chinese cohort of IN patients including 15 sporadic cases and 5 families with X-Linked IN. We performed molecular genetic analysis on *FRMD7* and *GPR143* in participants from these 20 unrelated patients to detect the causative mutations.

## MATERIALS AND METHODS

### Study subjects

This study received the approval of The Eye Hospital of Wenzhou Medical University and complied with the Declaration of Helsinki. Written informed consents were obtained from each patient. Twenty Chinese families exhibited X-Linked IN were recruited and peripheral blood samples were collected from some participants. We performed detailed ophthalmologic examinations on selected patients and also tested for visual acuities, slit lamp, intraocular pressure, fundoscopy and angle of head turn.

### DNA extraction

We selected all the twenty probands and some healthy family members from each family and extracted DNA from them using a DNA Extraction Kit (TIANGEN, Beijing) according to the manufacturer's instructions. DNA was quantified using Nanodrop 2000 (Thermal Fisher Scientific, DE).

**Mutation screening and data analysis**

Primers of all coding exons and exon-intron junctions of *FRMD7* and *GPR143* were designed. Polymerase chain reaction (PCR) and Sanger sequencing were utilized to amplify each exon of *FRMD7* and *GPR143* in each participant. Sequencing results were analyzed by Mutation Surveyor (Softgenetics, PA) and potential pathogenic effects of the mutations on protein function were estimated using MutationTaster (http://www.mutationtaster.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html). Co-segregation of putative causative variants were performed in the family members.

## RESULTS

**Clinical observations**

In total, twenty unrelated patients with IN were involved in this study. The inheritance pattern in the five families is consistent with an X-Linked mode of inheritance. The other fifteen patients do not have family histories, indicating all of them were simplex cases. All of the examined patients displayed symptoms of binocular involuntary horizontal eye movements with different intermediate frequencies. They also suffered from reduced visual acuity, amblyopia and astigmatism. No other anomalies like anterior eye segment, color vision and abnormalities in the optic nerves, were found.

**Mutation detection**

After Sanger sequencing and mutational analysis, we failed to find any putative mutations in the 15 sporadic cases. However, among the 5 families with XLIN, two

candidate mutations were identified. A novel missense mutation, c.1090C>A (p.Q364X), in exon 12 of *FRMD7*, which would lead to a stop codon at position 364, was detected by Sanger sequencing analysis of a patient (IV:1) from family F1 (Figure 1). All affected males in family F1 carried this mutation and the heterozygous female carriers showed no phenotypes of the disease. Therefore, the mutation completely segregated with the phenotype in the family F1 (Figure 2). Sequencing analysis of a patient (III:4) from family F2 (Figure 1) showed that he harbored a missense mutation (c.781C>G, p.R261G) in exon 10 of *FRMD7*, which would result in amino acid substitution of arginine by glutamate at the position of 261 (Figure 2). Protein prediction programs assigned this mutation as pathogenic and impactful of protein function. Notably, this mutation has previously been reported by other researchers.[14] This mutation also followed an X-Linked recessive inheritance. Both of mutations Q364X and R261G were located in highly conserved regions (Figure 3). There are five affected males in family F1 and two affected males in family F2. The age distribution of affected individuals ranged from 4 to 35 years and the age of onset was between 3 and 6 months of age (Table 1). Of note, none of the participants showed abnormalities in *GPR143* gene. Taken together, we conclude that the two variants, c.1090C>A (p.Q364X) and c.781C>G (p.R261G) in *FRDM7* gene, are the disease-causing mutations in the two Chinese families with XLIN.

## DISCUSSION

Infantile nystagmus (IN) is an inherited eye disease that is usually exhibits as spontaneous eye movement accompanied with some complications such as amblyopia, strabismus, torticollis, refractive error and lateral view. The genetic etiology of IN is not yet fully understood, especially the sporadic cases.[15] In this study, for the purpose

of identifying disease-causing genetic defects in IN population, we recruited twenty infantile nystagmus families, including fifteen sporadic cases and five X-Linked IN families. Since *FRMD7*and *GPR143* have been previously identified as the pathogenic genes of X-Linked IN, we focused on screening exons and exon/intron boundaries of the two genes using Sanger sequencing.[16] Up to now, more than 40 mutations in the *FRMD7* gene have been found which mainly concentrated in two key regions including FERM and FA domains.[17] However, the function of the protein still remains poorly understood. Previous studies demonstrated the role of *FRMD7* in the regulation of neuronal cytoskeletal dynamics at the growth cone through Rho GTPase signaling. Genetic defects in *FRMD7* may damage the recruitment and the activation of the Rho family of GTPases (Cdc42, Rac1, and RhoA) and their regulators.[18,19] Two mutations in *FRMD7* gene, including one novel nonsense mutation (c.1090C>A, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in this study. In family F1, the affected males harbored the novel nonsense mutation (c.1090C>A, p.Q364X) and all exhibited typical manifestations of IN. Because the females in family F1 that carried a heterozygous mutation showed no disease phenotypes, we conclude that the penetrance of the mutation in this family was 100%, which was consistent with IN diagnosis of the offsprings. Similar to that in family F1, the mutation (p.R261G) identified in family F2 was also recessive and has previously been reported in another Chinese X-Linked IN family. Taken together, we identified disease-causing mutations in two X-Linked IN families, with the detection rate of 40% (2/5). However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases, suggesting the very limited etiology contribution of *FRMD7* or *GPR143* in the simplex IN patients.

Taken together, we screened *FRMD7* and *GPR143* genes in fifteen patients with simplex IN and five probands with XLIN. Finally, two disease-causing mutations were identified in *FRMD7* gene in two XLIN families, with the detection rate of 40%. However, none candidate variants were discovered in any simplex IN patients. The results demonstrated that mutations in *FRMD7* appeared to be the major genetic cause of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

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**Contributions:** ZBJ and XPY conceived the idea, HC, XFH, HYY, LY, SZX and JC collected the samples and performed the experiments, XFH and FZ performed data analyses, XFH and ZBJ wrote the manuscript. All authors have read and approved the final manuscript.

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**Competing interests:** None.

**Patient consent:** Obtained.

**Ethics approval:** This study was conducted with the approval of the Eye Hospital of Wenzhou Medical University.

**Provenance and peer review:** Not commissioned; externally peer reviewed.

**Data sharing statement:** The original sequencing results are available on request.



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**Table 1. The clinical features of family F1 and F2.**

| Subject | Family | Age (y) | Sex | Onset age | BCVA (OD/OS) | Clinical findings |
|---|---|---|---|---|---|---|
| IV:1 | F1 | 2 | Male | 3 months | 0.3/0.3 | Horizontal nystagmus |
| III:3 | F1 | 27 | Male | 5 months | 0.2/0.3 | Horizontal nystagmus |
| II:5 | F1 | 45 | Female | - | 1.0/1.0 | Normal |
| II:10 | F1 | 38 | Female | - | 1.0/1.0 | Normal |
| III:4 | F2 | 12 | Male | 6 months | 0.3/0.2 | Horizontal nystagmus |
| III:5 | F2 | 7 | Female | - | 1.0/1.0 | Normal |
| III:6 | F2 | 4 | Male | 5 months | 0.2/0.2 | Horizontal nystagmus |
| II:6 | F2 | 31 | Female | - | 1.0/1.0 | Normal |

BCVA, best corrected visual acuity.

## Figure legends

**Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.** Filled symbols represent affected patients and unfilled symbols indicate unaffected subjects. The bars over the symbol indicate subjects enrolled in this study. Arrow marks the proband.

**Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.** (A)A single transition mutation was observed at position 1090 (C>A) of the *FRMD7* gene, causing a substitution of Gln by a stop codon at codon 364 (Q364X). (B)A single transition mutation was observed at position 781(C>G) of the *FRMD7* gene, causing a substitution of Arg by Gly at codon 261 (A261G).

**Figure 3. Multiple-sequence alignment of the *FRMD7* proteins from different species.** The red outline in the alignment shows the location of the mutations. Both of mutations Q364X and R261G were located in highly conserved regions.

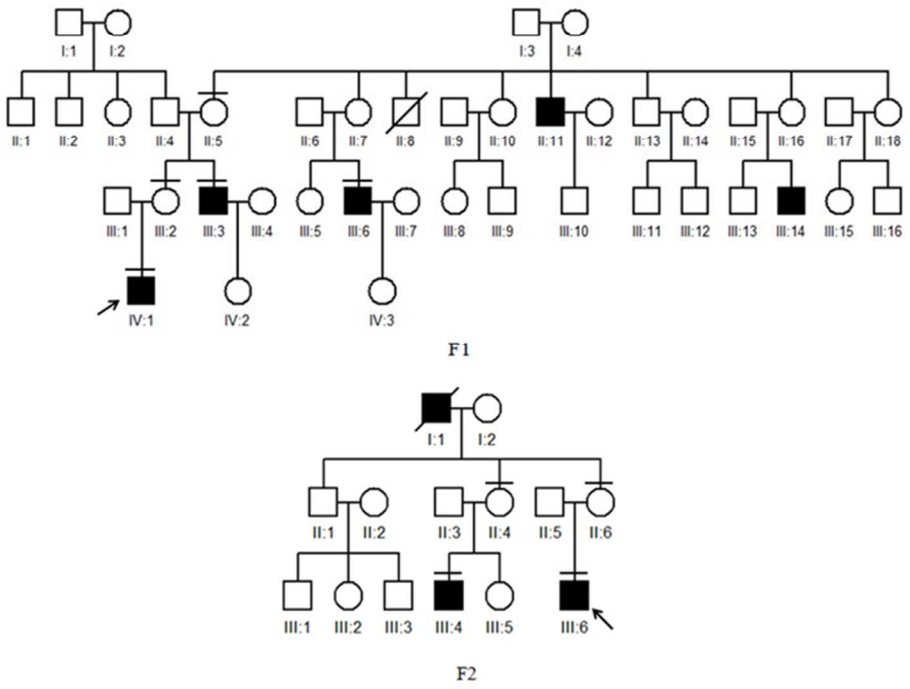


Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

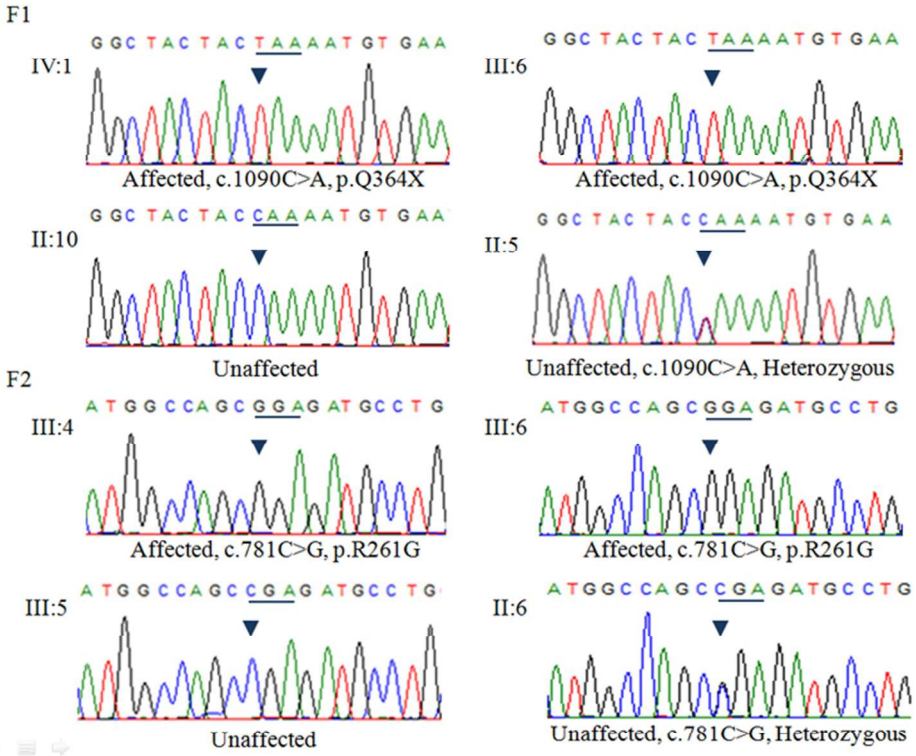


Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.
67x58mm (300 x 300 DPI)

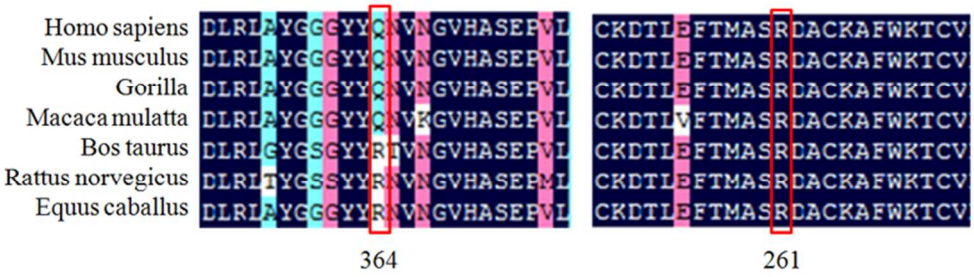


Figure 3. Multiple-sequence alignment of the FRMD7 proteins from different species.
67x32mm (300 x 300 DPI)

**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1,2 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1,2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3,4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 3,4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5,6 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5,6 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 5,6 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | 5,6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5,6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5,6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | |
| Study size | 10 | Explain how the study size was arrived at | |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | |
| | | (*b*) Describe any methods used to examine subgroups and interactions | |
| | | (*c*) Explain how missing data were addressed | |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | |
| **Results** | | | |

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 6,7 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 6,7 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| | | (c) Summarise follow-up time (eg, average and total amount) | |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 6,7 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 7-9 |
| **Limitations** | | | 7-9 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 7-9 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 9 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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**Results:** Two mutations in *FRMD7* gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in two X-Linked IN families, with the detection rate of 40% (2/5). However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases.

**Conclusions:** In conclusion, the results suggested that mutations in *FRMD7* appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

**Strengths and limitations of this study:**

- As very few studies focused on the sporadic cases, the aim of our study is to detect the genetic basis of both sporadic IN and X-Linked IN.
- Two mutations in FRMD7 gene, including one novel nonsense mutation, were identified in two X-Linked IN families.
- The results suggested that mutations in FRMD7 appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. More samples are required in the future study.

## INTRODUCTION

Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes involuntary, rapid and repetitive movements of the eyes. Signs of IN usually appear at birth or develop within the first six months of life. The incidence of all forms of infantile nystagmus is estimated to be 14 per 10,000 individuals.[1] The most common manifestation of IN is visual acuity impairment, which is caused by excessive motion of images on the retina or by the movement of images away from the fovea. The abnormal eye movement sometimes become worse when an affected person stares at an object or is feeling anxious. The cerebellum is thought to have a key role to ocular motor control.[2] Patients' eye oscillations can be horizontal, vertical, torsional, or combination of the three, and horizontal is the most common. To date, the pathogenesis of IN remains unclear. Many researchers attribute the disease to the abnormal control of the part of the brain that is in charge of ocular motor.[3] Currently, there is no effective treatment for IN, only very limited surgical, optical or

pharmaceutical therapies have been employed to improve nystagmus waveforms and visual acuity.[4,5] With the exception of idiopathic infantile nystagmus, IN can be complicated by other diseases like albinism, achromatopsia, Leber congenital amaurosis and visual deprivation at early age. It present as a component of other neurological syndromes and neurologic diseases.[6]

Infantile nystagmus displayed extreme genetic and clinical heterogeneity, and the most common form of IN follows that of an X-Linked pattern. To date, two major genes have been defined as the causative genes of hereditary X-Linked infantile nystagmus (XLIN).[7] The *FRMD7* gene contains an N-terminal FERM domain with high conservation and FERM-adjacent domain without significant homology. Mutations in *FRMD7* gene is currently the most common cause of XLIN.[8] It is mainly expressed in the retina and in parts of the brain that coordinate eye movement, like the cerebellum and the lateral ventricles.[9] Although the exact function of this gene is still unclear, previous research suggests that *FRMD7* gene mutations may lead to nystagmus by disrupting the normal development of certain nerve cells in the brain and the retina. Most studies focus on its N-terminal FERM domain which may play a role between the plasma and actin cytoskeleton. Previous studies also suggest a link between membrane extension during neuronal development and remodeling of the actin cytoskeleton. The other disease-causing gene of XLIN, *GPR143*, encodes a protein that binds to heterotrimeric G proteins and affects pigment production in the iris, retinal pigment epithelium and skin.[10] This protein is thought to be involved in intracellular signal transduction. Mutations in *GPR143* have also been shown to cause X-Linked ocular albinism type 1(OA1), which is a multi-symptom syndrome that could lead to reduced visual acuity, nystagmus, strabismus and so on.[11] However, a deletion mutation of *GPR143* gene has also been reported in a Chinese IN family

without typical phenotype of ocular albinism.[12] Nearly half of X-Linked IN families have genetic defects in the *FRMD7* gene. However, very limited studies investigate the genetic basis of sporadic cases with IN.[13]

In this study, we recruited a Chinese cohort of IN patients including 15 sporadic cases and 5 families with X-Linked IN. We performed molecular genetic analysis on *FRMD7* and *GPR143* in participants from these 20 unrelated patients to detect the causative mutations.

## MATERIALS AND METHODS

### Study subjects

This study received the approval of The Eye Hospital of Wenzhou Medical University and complied with the Declaration of Helsinki. Written informed consents were obtained from each patient. Twenty Chinese families with idiopathic IN were recruited and peripheral blood samples were collected from some participants. We performed detailed ophthalmologic examinations on selected patients and also tested for visual acuities, slit lamp, intraocular pressure, fundoscopy and angle of head turn.

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We selected all the twenty probands and some healthy family members from each family and extracted DNA from them using a DNA Extraction Kit (TIANGEN, Beijing) according to the manufacturer's instructions. DNA was quantified using Nanodrop 2000 (Thermal Fisher Scientific, DE).

### Mutation screening and data analysis

Primers of all coding exons and exon-intron junctions of *FRMD7* and *GPR143* were according to the previous studies.[14,15] Polymerase chain reaction (PCR) and Sanger sequencing were utilized to amplify each exon of *FRMD7* and *GPR143* in each participant. Sequencing results were analyzed by Mutation Surveyor (Softgenetics, PA) and potential pathogenic effects of the mutations on protein function were estimated using MutationTaster (http://www.mutationtaster.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html). The annotation of frequency was according to the 1000 Genomes (http://www.1000genomes.org/). Co-segregation of putative causative variants were performed in the family members.

## RESULTS

### Clinical observations

In total, twenty unrelated patients with IN were involved in this study. The inheritance pattern in the five families is consistent with an X-Linked mode of inheritance. The other fifteen patients do not have family histories, indicating all of them were simplex cases. All of the examined patients displayed symptoms of binocular involuntary horizontal eye movements with different intermediate frequencies. They also suffered from reduced visual acuity, amblyopia and astigmatism. No other anomalies like anterior eye segment, color vision and abnormalities in the optic nerves, were found.

### Mutation detection

After Sanger sequencing and mutational analysis, we found that five probands carried DNA variants in *FRMD7* (Table 1). However, three variants were considered as polymorphisms because the frequency was >0.01 and the predicted functional effects

displayed tolerate. Finally, two pathogenic mutations were identified among the 5 families with XLIN (Table 1). A novel nonsense mutation, c.1090C>T (p.Q364X), in exon 12 of *FRMD7*, which would lead to a stop codon at position 364, was detected by Sanger sequencing analysis of a patient (IV:1) from family F1 (Figure 1). Sequencing analysis of a patient (III:4) from family F2 (Figure 1) showed that he harbored a missense mutation (c.781C>G, p.R261G) in exon 10 of *FRMD7*, which would result in amino acid substitution of arginine by glutamate at the position of 261 (Figure 2). All protein prediction programs assigned this mutation as damaging and impactful of protein function. Notably, this mutation has previously been reported by other researchers.[16] This mutation also followed an X-Linked recessive inheritance. Both of mutations Q364X and R261G were located in highly conserved regions (Figure 3). There are five affected males in family F1 and two affected males in family F2. The age distribution of affected individuals ranged from 4 to 35 years and the age of onset was between 3 and 6 months of age (Table 2). Of note, none of the participants showed abnormalities in *GPR143* gene. Taken together, we conclude that the two variants, c.1090C>T (p.Q364X) and c.781C>G (p.R261G) in *FRDM7* gene, are the disease-causing mutations in the two Chinese families with XLIN.

## DISCUSSION

The genetic etiology of IN is not yet fully understood, especially the sporadic cases.[17] In this study, we recruited twenty infantile nystagmus families, including fifteen sporadic cases and five X-Linked IN families. The major disease-causing genes, *FRMD7*and *GPR143* have been screened.

Up to now, more than 40 mutations in the *FRMD7* gene have been found which mainly concentrated in two key regions including FERM and FA domains.[18,19]

However, the function of the protein still remains poorly understood. Previous studies demonstrated the role of *FRMD7* in the regulation of neuronal cytoskeletal dynamics at the growth cone through Rho GTPase signaling.[20,21] Two mutations in *FRMD7* gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in this study. In family F1, the affected males harbored the novel nonsense mutation (c.1090C>T, p.Q364X) and all exhibited typical manifestations of IN. The mutation (p.R261G) identified in family F2 was also recessive and has previously been reported in another Chinese X-Linked IN family. Taken together, we identified disease-causing mutations in two X-Linked IN families, with the detection rate of 40% (2/5). The pedigrees of other three X-Linked IN families were displayed in the Figure S1. However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases, suggesting the very limited etiology contribution of *FRMD7* or *GPR143* in the simplex IN patients.

Taken together, we screened *FRMD7* and *GPR143* genes in fifteen patients with simplex IN and five probands with XLIN. Finally, two disease-causing mutations were identified in *FRMD7* gene in two XLIN families, with the detection rate of 40%. However, none candidate variants were discovered in any simplex IN patients. The results demonstrated that mutations in *FRMD7* appeared to be the major genetic cause of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

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**Contributions:** ZBJ and XPY conceived the idea, HC, XFH, HYY, LY, SZX and JC collected the samples and performed the experiments, XFH and FZ performed data analyses, XFH and ZBJ wrote the manuscript. All authors have read and approved the final manuscript.

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**Competing interests:** None.

**Patient consent:** Obtained.

**Ethics approval:** This study was conducted with the approval of the Eye Hospital of Wenzhou Medical University.

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**Data sharing statement:** No additional data available.

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**Table 1. The summary of variants.**

| Family | Proband | Gene | Variant | Type | Frequency* | Predicted effects# |
|---|---|---|---|---|---|---|
| F1 | Male | *FRMD7* | c.1090C>T (p.Q364X) | Hemizygous | None | Damaging |
| F2 | Male | *FRMD7* | c.781C>G (p.R261G) | Hemizygous | None | Damaging |
| F6 | Female | *FRMD7* | c.1403G>A (p.R468H) | Heterozygous | 0.07 | Tolerated |
| F7 | Female | *FRMD7* | c.1403G>A (p.R468H) | Heterozygous | 0.07 | Tolerated |
| F20 | Female | *FRMD7* | c.1533T>C (No change) | Heterozygous | 0.26 | Tolerated |

* Data from 1000 Genomes. # Predicted by SIFT.

**Table 2. The clinical features of family with XLIN.**

| Subject | Family | Age (y) | Sex | Onset age | BCVA (OD/OS) | Clinical findings |
|---|---|---|---|---|---|---|
| IV:1 | F1 | 2 | Male | 3 months | 0.3/0.3 | Horizontal nystagmus |
| III:3 | F1 | 27 | Male | 5 months | 0.2/0.3 | Horizontal nystagmus |
| II:5 | F1 | 45 | Female | - | 1.0/1.0 | Normal |
| II:10 | F1 | 38 | Female | - | 1.0/1.0 | Normal |
| III:4 | F2 | 12 | Male | 6 months | 0.3/0.2 | Horizontal nystagmus |
| III:5 | F2 | 7 | Female | - | 1.0/1.0 | Normal |
| III:6 | F2 | 4 | Male | 5 months | 0.2/0.2 | Horizontal nystagmus |
| II:6 | F2 | 31 | Female | - | 1.0/1.0 | Normal |
| II:5 | F3 | 36 | Male | 5 months | 0.1/0.1 | Horizontal nystagmus |
| III:5 | F4 | 8 | Male | 6 months | 0.3/0.3 | Horizontal nystagmus |
| III:3 | F5 | 37 | Male | 8 months | 0.2/0.2 | Horizontal nystagmus |

BCVA, best corrected visual acuity.

## Figure legends

**Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.** Filled symbols represent affected patients and unfilled symbols indicate unaffected subjects. The bars over the symbol indicate subjects enrolled in this study. Arrow marks the proband.

**Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.** (A)A single transition mutation was observed at position 1090 (C>T) of the *FRMD7* gene, causing a substitution of Gln by a stop codon at codon 364 (Q364X). (B)A single transition mutation was observed at position 781(C>G) of the *FRMD7* gene, causing a substitution of Arg by Gly at codon 261 (A261G).

**Figure 3. Multiple-sequence alignment of the *FRMD7* proteins from different species.** The red outline in the alignment shows the location of the mutations. Both of mutations Q364X and R261G were located in highly conserved regions.

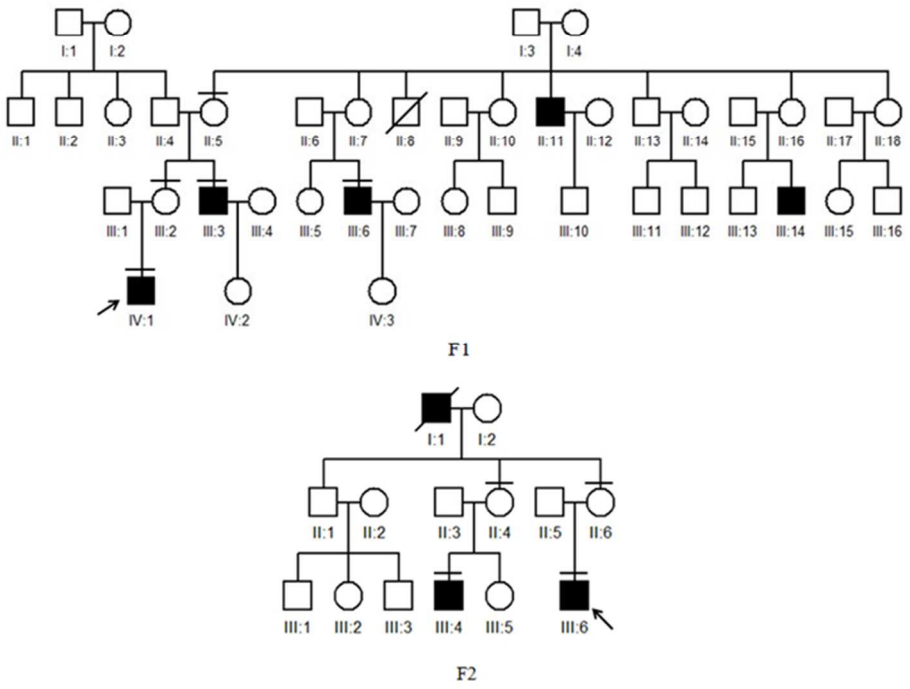



Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

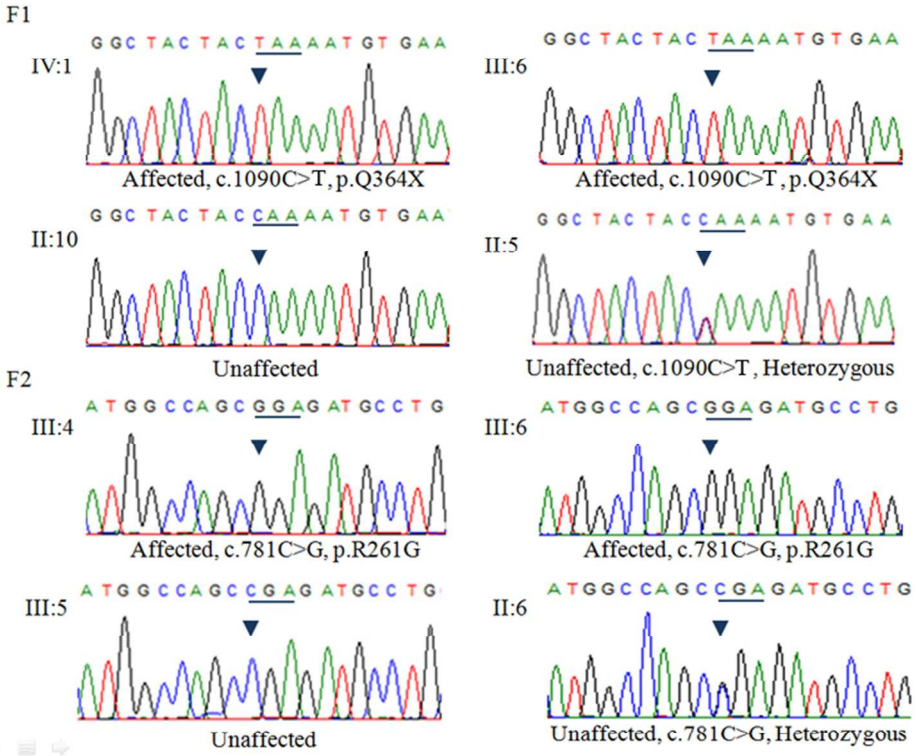
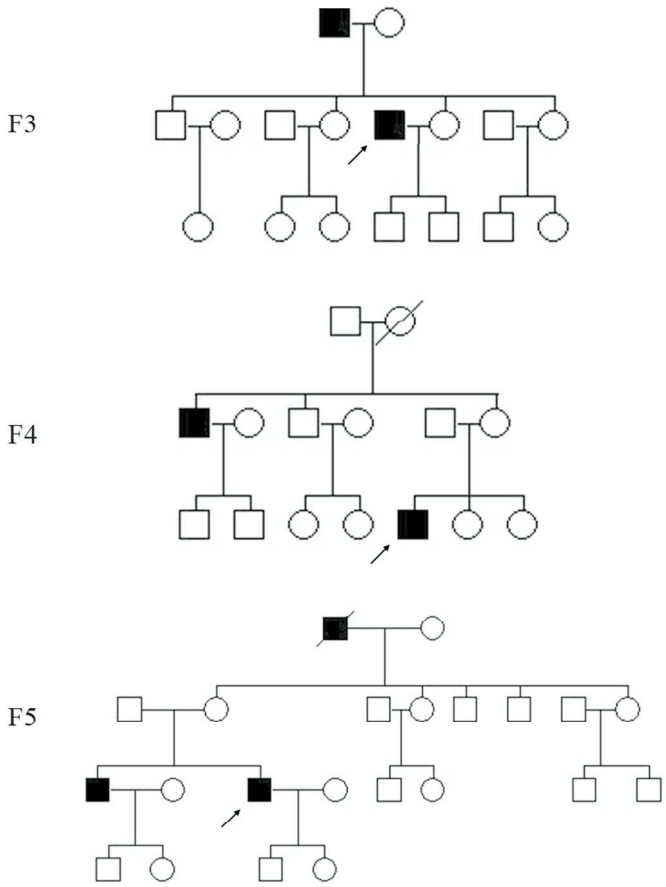



Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.
67x58mm (300 x 300 DPI)


Figure 3. Multiple-sequence alignment of the FRMD7 proteins from different species.
67x32mm (300 x 300 DPI)

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# Molecular genetic analysis of patients with sporadic and X-Linked infantile nystagmus

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**Key words**: infantile nystagmus; *FRMD7* gene; novel mutations; sporadic; X-Linked

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## ABSTRACT

**Objectives:** Infantile nystagmus (IN) is a genetically heterogeneous condition characterized by involuntary rhythmic oscillations of eyes accompanied with vision impairment of different severity. Two genes have been identified as major disease-causing genes for IN, *FRMD7* and *GPR143*. The aim of our study was to detect the genetic basis of both sporadic IN and X-Linked IN.

**Design:** Prospective analysis.

**Patients:** Twenty Chinese patients underwent molecular genetic analysis, including fifteen sporadic IN cases and five X-Linked IN families, were recruited in this study. We first performed PCR-based DNA sequencing of the entire coding region and the splice junctions of the two genes mentioned above in the recruited individuals from the two pedigrees. Then mutational analysis and co-segregation confirmation were performed.

**Setting:** All clinical examinations and genetic experiments were performed in the Eye Hospital of Wenzhou Medical University.

**Results:** Two mutations in *FRMD7* gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in two X-Linked IN families, with a detection rate of 40% (2/5). However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases.

**Conclusions:** In summary, the results suggest that mutations in *FRMD7* appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

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- As very few studies focused on the sporadic cases, the aim of our study was to detect the genetic basis of both sporadic IN and X-Linked IN.
- Two mutations in FRMD7 gene, including one novel nonsense mutation, were identified in two X-Linked IN families.
- The results suggest that mutations in FRMD7 appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. More samples are required in future study.

## INTRODUCTION

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IN displays extreme genetic and clinical heterogeneity, and the most common form of IN follows an X-Linked pattern. To date, two major genes *FRMD7* and *GPR143* have been identified as the causative genes of hereditary X-Linked infantile nystagmus (XLIN).[7] The *FRMD7* gene contains an N-terminal FERM (F for 4.1 protein, E for ezrin, R for radixin and M for moesin) domain with high conservation and a FERM-adjacent domain without significant homology. Mutations in *FRMD7* gene are currently the most common cause of XLIN.[8] It is mainly expressed in the

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## RESULTS

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### Mutation detection

After Sanger sequencing and mutational analysis, we found that five probands carried DNA variants in *FRMD7* (Table 1). However, three variants were considered as polymorphisms because the frequency was >0.01 and the predicted functional effects displayed tolerance. Finally, two pathogenic mutations were identified among the five families with XLIN (Table 1). A novel nonsense mutation, c.1090C>T (p.Q364X), in exon 12 of *FRMD7*, which would lead to a stop codon at position 364, was detected by Sanger sequencing analysis of a patient (IV:1) from family F1 (Figure 1). Sequencing analysis of a patient (III:4) from family F2 (Figure 1) showed that he harbored a missense mutation (c.781C>G, p.R261G) in exon 10 of *FRMD7*, which would result in amino acid substitution of arginine by glutamate at the position of 261 (Figure 2). All protein prediction programs assigned this mutation as damaging and impactful protein function. Notably, this mutation has previously been reported by other researchers.[16] This mutation also followed an X-Linked recessive inheritance.

Both of mutations Q364X and R261G were located in highly conserved regions (Figure 3). There are five affected males in family F1 and two affected males in family F2. The age distribution of the affected individuals ranged from 4 to 35 years and the age of onset was between 3 and 6 months of age (Table 2). Of note, none of the participants showed abnormalities in *GPR143* gene. Taken together, we conclude that the two variants, c.1090C>T (p.Q364X) and c.781C>G (p.R261G) in *FRDM7* gene, are the disease-causing mutations in the two Chinese families with XLIN.

## DISCUSSION

The genetic etiology of IN is not yet fully understood, especially the sporadic cases.[17] In this study, we recruited twenty IN families, including fifteen sporadic cases and five X-Linked IN families. The major disease-causing genes, *FRMD7*and *GPR143* have been screened.

Up to now, more than 40 mutations have been found in the *FRMD7* gene which mainly concentrated in two key regions: FERM and FA domains.[18,19] However, the function of the protein still remains poorly understood. Previous studies demonstrated the role of *FRMD7* in the regulation of neuronal cytoskeletal dynamics at the growth cone through Rho GTPase signaling.[20,21] Two mutations in the *FRMD7* gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified in this study. In family F1, the affected males harbored the novel nonsense mutation (c.1090C>T, p.Q364X) and all exhibited typical manifestations of IN. The mutation (p.R261G) identified in family F2 was also recessive and has previously been reported in another Chinese X-Linked IN family. Taken together, we identified disease-causing mutations in two X-Linked IN families, with a detection rate of 40% (2/5). The pedigrees of other three

X-Linked IN families are shown in Figure S1. However, none of putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases, suggesting very limited etiology contribution of *FRMD7* or *GPR143* in the simplex IN patients.

Taken together, we screened *FRMD7* and *GPR143* genes in fifteen patients with simplex IN and five probands with XLIN. Finally, two disease-causing mutations were identified in *FRMD7* gene in two XLIN families, with a detection rate of 40%. However, none candidate variants were discovered in any simplex IN patients. The results demonstrated that the mutations in *FRMD7* appeared to be the major genetic cause of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our findings provide further insights into the mutation spectrum of *FRMD7*, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

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**Contributions:** ZBJ, HZ and XPY conceived the idea, WLD, XLL, DJX, HYY, LY, SZX and XC collected the samples and performed the experiments, XFH and FZ performed data analyses, XFH and ZBJ wrote the manuscript. All authors have read and approved the final manuscript.

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**Patient consent:** Obtained.

**Ethics approval:** This study was conducted with the approval of the Eye Hospital of Wenzhou Medical University.

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**Data sharing statement:** No additional data available.

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**Table 1. The summary of variants.**

| Family | Proband | Gene | Variant | Type | Frequency* | Predicted effects# |
|---|---|---|---|---|---|---|
| F1 | Male | *FRMD7* | c.1090C>T (p.Q364X) | Hemizygous | None | Damaging |
| F2 | Male | *FRMD7* | c.781C>G (p.R261G) | Hemizygous | None | Damaging |
| F6 | Female | *FRMD7* | c.1403G>A (p.R468H) | Heterozygous | 0.07 | Tolerated |
| F7 | Female | *FRMD7* | c.1403G>A (p.R468H) | Heterozygous | 0.07 | Tolerated |
| F20 | Female | *FRMD7* | c.1533T>C (No change) | Heterozygous | 0.26 | Tolerated |

* Data from 1000 Genomes. # Predicted by SIFT.

**Table 2. The clinical features of family with XLIN.**

| Subject | Family | Age (y) | Sex | Onset age | BCVA (OD/OS) | Clinical findings |
|---|---|---|---|---|---|---|
| IV:1 | F1 | 2 | Male | 3 months | 0.3/0.3 | Horizontal nystagmus |
| III:3 | F1 | 27 | Male | 5 months | 0.2/0.3 | Horizontal nystagmus |
| II:5 | F1 | 45 | Female | - | 1.0/1.0 | Normal |
| II:10 | F1 | 38 | Female | - | 1.0/1.0 | Normal |
| III:4 | F2 | 12 | Male | 6 months | 0.3/0.2 | Horizontal nystagmus |
| III:5 | F2 | 7 | Female | - | 1.0/1.0 | Normal |
| III:6 | F2 | 4 | Male | 5 months | 0.2/0.2 | Horizontal nystagmus |
| II:6 | F2 | 31 | Female | - | 1.0/1.0 | Normal |
| II:5 | F3 | 36 | Male | 5 months | 0.1/0.1 | Horizontal nystagmus |
| III:5 | F4 | 8 | Male | 6 months | 0.3/0.3 | Horizontal nystagmus |
| III:3 | F5 | 37 | Male | 8 months | 0.2/0.2 | Horizontal nystagmus |

BCVA, best corrected visual acuity.

## Figure legends

**Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.** Filled symbols represent affected patients and unfilled symbols indicate unaffected subjects. The bars over the symbol indicate subjects enrolled in this study. Arrow marks the proband.

**Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.** (A)A single transition mutation was observed at position 1090 (C>T) of the *FRMD7* gene, causing a substitution of Gln by a stop codon at codon 364 (Q364X). (B)A single transition mutation was observed at position 781(C>G) of the *FRMD7* gene, causing a substitution of Arg by Gly at codon 261 (A261G).

**Figure 3. Multiple-sequence alignment of the *FRMD7* proteins from different species.** The red outline in the alignment shows the location of the mutations. Both of mutations Q364X and R261G are located in highly conserved regions.

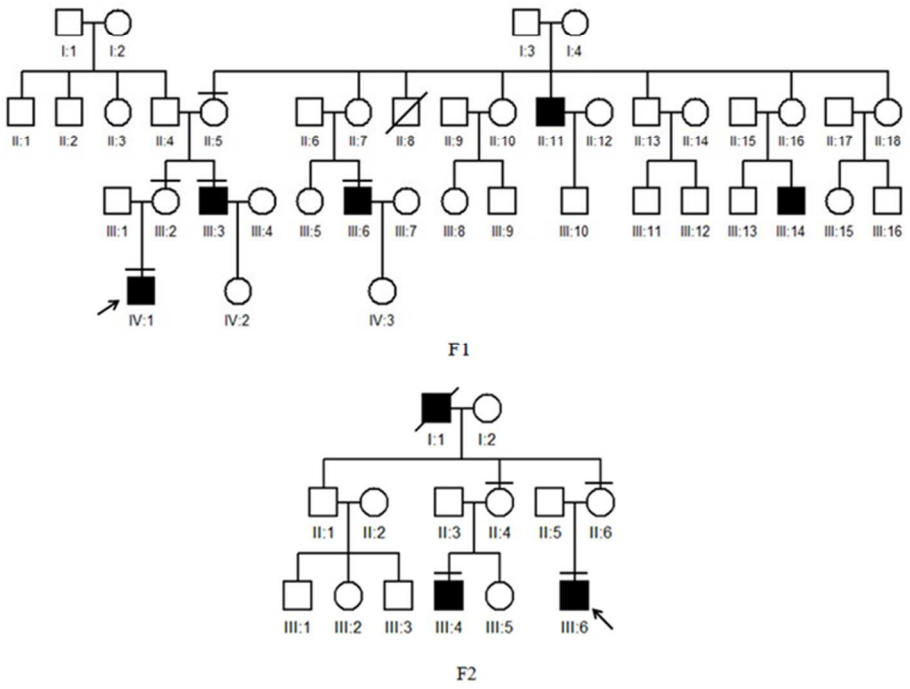


Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

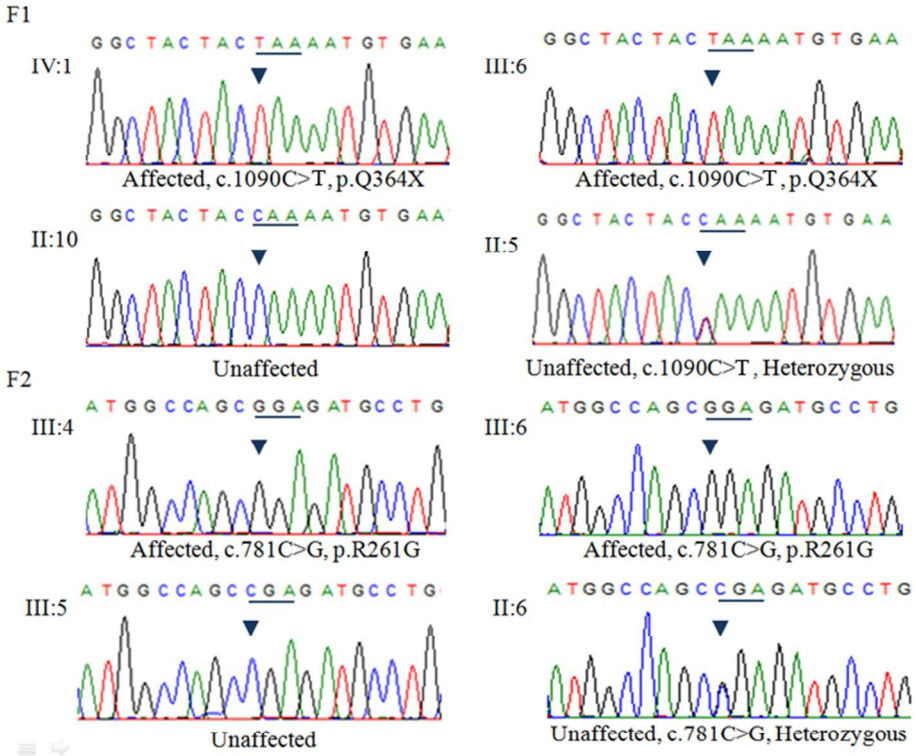


Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1and F2.
67x58mm (300 x 300 DPI)


Figure 3. Multiple-sequence alignment of the FRMD7 proteins from different species.
67x32mm (300 x 300 DPI)