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

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Keywords:	infantile nystagmus, FRMD7 gene, novel mutations, sporadic, X-Linked

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Manuscripts

1 1 **Molecular genetic analysis of patients with sporadic and X-Linked infantile**
2 2 **nystagmus**

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32 16
33 17 **Key words:** infantile nystagmus; *FRMD7* gene; novel mutations; sporadic; X-Linked

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35 19 **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,

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3 76 optical or pharmaceutical therapies have been employed to improve nystagmus
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5 77 waveforms and vision acuity.^{4,5} With the exception of idiopathic infantile nystagmus,
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7 78 IN can be complicated by other diseases like albinism, achromatopsia, Leber
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10 79 congenital amaurosis and visual deprivation at early age. It present as a component of
11
12 80 other neurological syndromes and neurologic diseases.⁶

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14 Infantile nystagmus can be inherited as an X-Linked, an autosomal recessive or an
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16 82 autosomal dominant disease with incomplete penetrance and variable expressivity.
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18 83 Although the inheritance pattern is heterogeneous, the most common form of IN
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20 84 follows that of an X-Linked pattern. To date, two major genes have been defined as
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22 85 the causative genes of hereditary X-Linked infantile nystagmus (XLIN).⁷ The *FRMD7*
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24 86 gene contains an N-terminal FERM domain with high conservation and FERM-
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26 87 adjacent domain without significant homology. Mutations in *FRMD7* gene is
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28 88 currently the most common cause of XLIN. It is mainly expressed in the retina and in
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30 89 parts of the brain that coordinate eye movement, like the cerebellum and the lateral
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32 90 ventricles.⁸ Although the exact function of this gene is still unclear, previous research
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34 91 suggests that *FRMD7* gene mutations may lead to nystagmus by disrupting the normal
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36 92 development of certain nerve cells in the brain and the retina. Most studies focus on
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38 93 its N-terminal FERM domain which may play a role between the plasma and actin
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40 94 cytoskeleton. Previous studies also suggest a link between membrane extension
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42 95 during neuronal development and remodeling of the actin cytoskeleton.⁹ The other
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44 96 disease-causing gene of XLIN, *GPR143*, encodes a protein that binds to
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46 97 heterotrimeric G proteins and affects pigment production in the iris, retinal pigment
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48 98 epithelium and skin.¹⁰ This protein is thought to be involved in intracellular signal
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50 99 transduction. Mutations in *GPR143* have been shown to cause X-Linked ocular
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52 100 albinism type 1(OA1), which is a multi-symptom syndrome that could lead to reduced
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3 101 visual acuity, nystagmus, strabismus and so on.^{11,12} However, a deletion mutation of
4
5 102 *GPR143* gene has also been reported in a Chinese IN family without typical
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7 103 phenotype of ocular albinism.¹³ Nearly half of X-Linked IN families have genetic
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9 104 defects in the *FRMD7* gene. However, very limited studies investigate the genetic
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11 105 basis of sporadic cases with IN.

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14 106 In this study, we recruited a Chinese cohort of IN patients including 15 sporadic
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16 107 cases and 5 families with X-Linked IN. We performed molecular genetic analysis on
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18 108 *FRMD7* and *GPR143* in participants from these 20 unrelated patients to detect the
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20 109 causative mutations.

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25 111 **MATERIALS AND METHODS**

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27 112 **Study subjects**

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29 113 This study received the approval of The Eye Hospital of Wenzhou Medical
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31 114 University and complied with the Declaration of Helsinki. Written informed consents
32
33 115 were obtained from each patient. Twenty Chinese families exhibited X-Linked IN
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35 116 were recruited and peripheral blood samples were collected from some participants.
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37 117 We performed detailed ophthalmologic examinations on selected patients and also
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39 118 tested for visual acuities, slit lamp, intraocular pressure, funduscopy and angle of head
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41 119 turn.

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47 121 **DNA extraction**

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49 122 We selected all the twenty probands and some healthy family members from each
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51 123 family and extracted DNA from them using a DNA Extraction Kit (TIANGEN,
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53 124 Beijing) according to the manufacturer's instructions. DNA was quantified using
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55 125 Nanodrop 2000 (Thermal Fisher Scientific, DE).

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127 **Mutation screening and data analysis**

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

137

138 **RESULTS**

139 **Clinical observations**

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

147

148 **Mutation detection**

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

151 candidate mutations were identified. A novel missense mutation, c.1090C>A
152 (p.Q364X), in exon 12 of *FRMD7*, which would lead to a stop codon at position 364,
153 was detected by Sanger sequencing analysis of a patient (IV:1) from family F1
154 (Figure 1). All affected males in family F1 carried this mutation and the heterozygous
155 female carriers showed no phenotypes of the disease. Therefore, the mutation
156 completely segregated with the phenotype in the family F1 (Figure 2). Sequencing
157 analysis of a patient (III:4) from family F2 (Figure 1) showed that he harbored a
158 missense mutation (c.781C>G, p.R261G) in exon 10 of *FRMD7*, which would result
159 in amino acid substitution of arginine by glutamate at the position of 261 (Figure 2).
160 Protein prediction programs assigned this mutation as pathogenic and impactful of
161 protein function. Notably, this mutation has previously been reported by other
162 researchers.¹⁴ This mutation also followed an X-Linked recessive inheritance. Both of
163 mutations Q364X and R261G were located in highly conserved regions (Figure 3).
164 There are five affected males in family F1 and two affected males in family F2. The
165 age distribution of affected individuals ranged from 4 to 35 years and the age of onset
166 was between 3 and 6 months of age (Table 1). Of note, none of the participants
167 showed abnormalities in *GPRI43* gene. Taken together, we conclude that the two
168 variants, c.1090C>A (p.Q364X) and c.781C>G (p.R261G) in *FRDM7* gene, are the
169 disease-causing mutations in the two Chinese families with XLIN.

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171 **DISCUSSION**

172 Infantile nystagmus (IN) is an inherited eye disease that is usually exhibits as
173 spontaneous eye movement accompanied with some complications such as amblyopia,
174 strabismus, torticollis, refractive error and lateral view. The genetic etiology of IN is
175 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.

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

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211 participation in this study.

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

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224 **Competing interests:** None.

225

226 **Patient consent:** Obtained.

227

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

230

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

232

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

234

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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 F1 and 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.

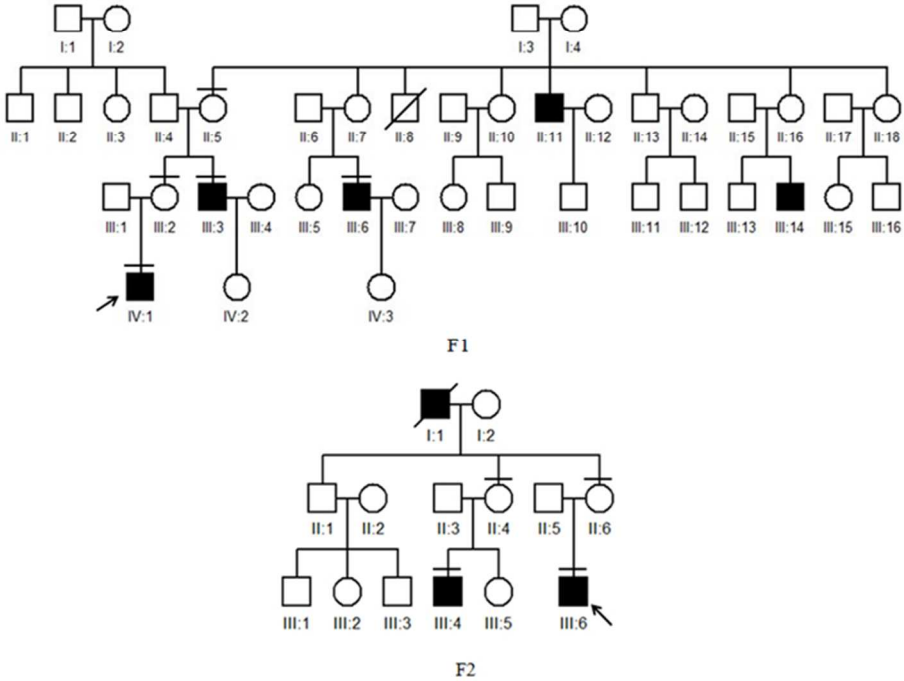


Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

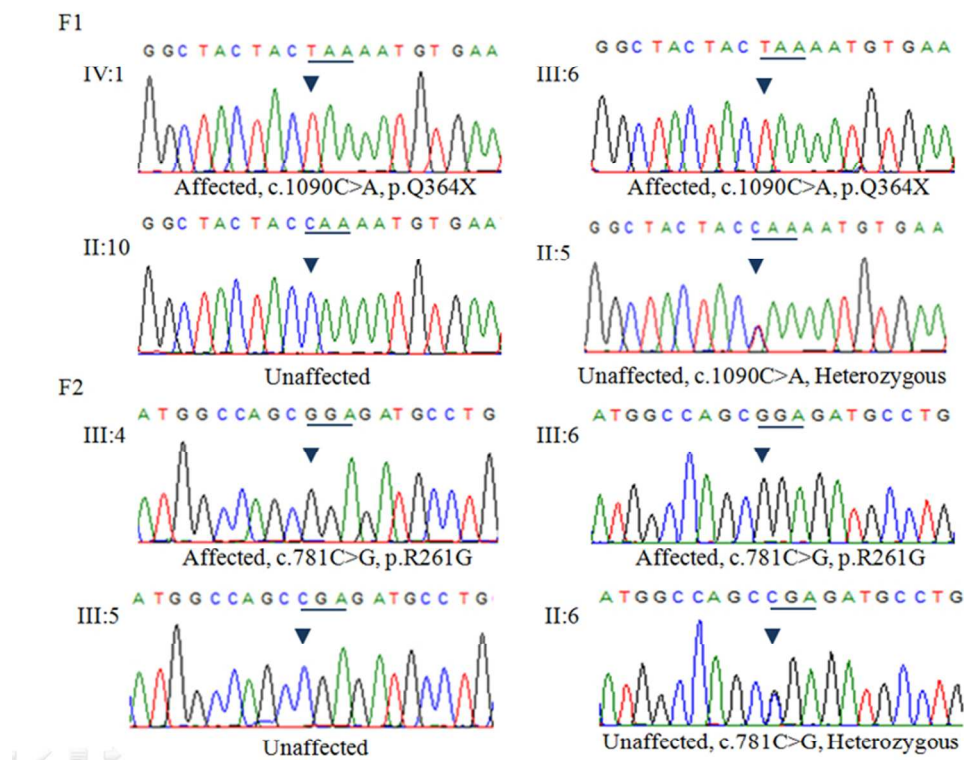


Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1 and F2. 67x58mm (300 x 300 DPI)



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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2 nystagmus

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

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19 **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>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

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

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

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109 **MATERIALS AND METHODS**

110 **Study subjects**

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

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118 **DNA extraction**

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

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124 **Mutation screening and data analysis**

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

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136 RESULTS

137 Clinical observations

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

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146 Mutation detection

147 After Sanger sequencing and mutational analysis, we found that five probands carried
148 DNA variants in *FRMD7* (Table 1). However, three variants were considered as
149 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 *GPRI43* 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 *GPRI43* 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}

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3 175 However, the function of the protein still remains poorly understood. Previous studies
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5 176 demonstrated the role of *FRMD7* in the regulation of neuronal cytoskeletal dynamics
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7 177 at the growth cone through Rho GTPase signaling.^{20,21} Two mutations in *FRMD7*
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9 178 gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one
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11 179 reported missense mutation (c.781C>G, p.R261G), were identified in this study. In
12
13 180 family F1, the affected males harbored the novel nonsense mutation (c.1090C>T,
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15 181 p.Q364X) and all exhibited typical manifestations of IN. The mutation (p.R261G)
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17 182 identified in family F2 was also recessive and has previously been reported in another
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19 183 Chinese X-Linked IN family. Taken together, we identified disease-causing mutations
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21 184 in two X-Linked IN families, with the detection rate of 40% (2/5). The pedigrees of
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23 185 other three X-Linked IN families were displayed in the Figure S1. However, none of
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25 186 putative mutations were identified in *FRMD7* or *GPR143* in any sporadic cases,
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27 187 suggesting the very limited etiology contribution of *FRMD7* or *GPR143* in the
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29 188 simplex IN patients.

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34 189 Taken together, we screened *FRMD7* and *GPR143* genes in fifteen patients with
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36 190 simplex IN and five probands with XLIN. Finally, two disease-causing mutations
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38 191 were identified in *FRMD7* gene in two XLIN families, with the detection rate of 40%.
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40 192 However, none candidate variants were discovered in any simplex IN patients. The
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42 193 results demonstrated that mutations in *FRMD7* appeared to be the major genetic cause
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44 194 of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our
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46 195 findings provide further insights into the mutation spectrum of *FRMD7*, which could
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48 196 be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus
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50 197 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.

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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 F1 and 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.

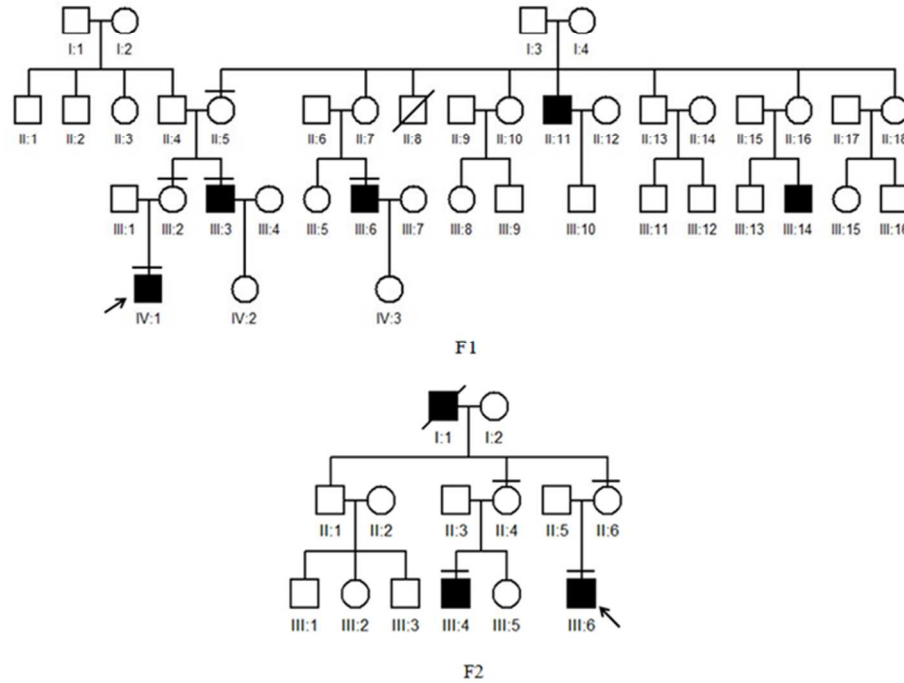


Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

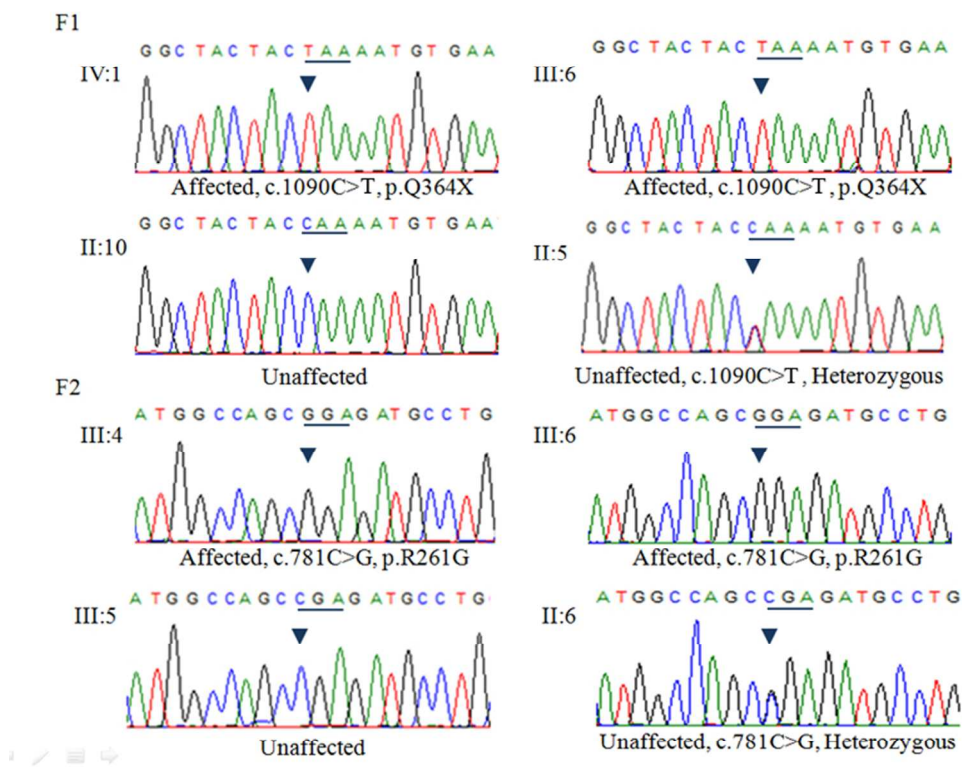


Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1 and F2. 67x58mm (300 x 300 DPI)

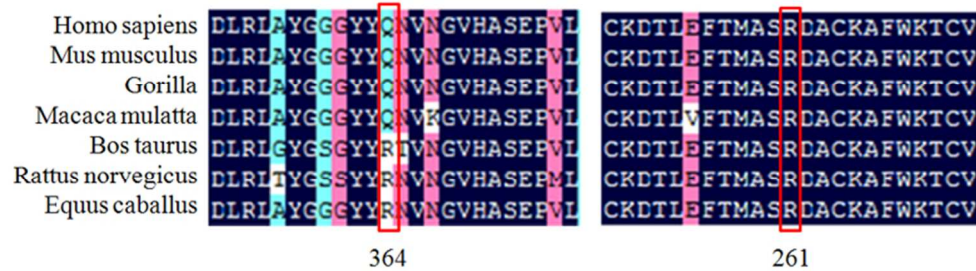
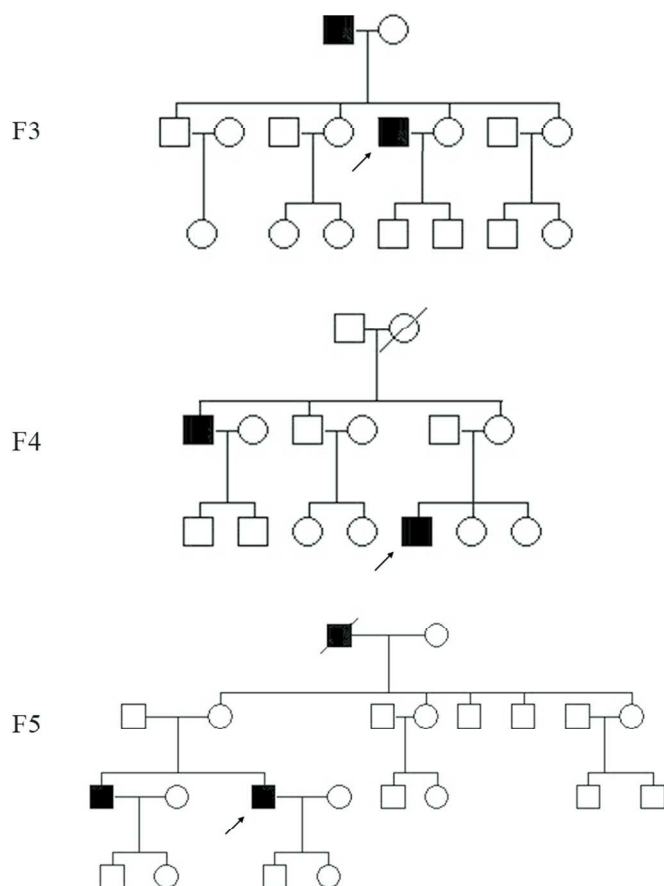


Figure 3. Multiple-sequence alignment of the FRMD7 proteins from different species.
67x32mm (300 x 300 DPI)



101x101mm (300 x 300 DPI)

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

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Secondary Subject Heading:	Genetics and genomics
Keywords:	nystagmus, FRMD7 gene, novel mutations

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Manuscripts

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 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.

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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.

51 **Strengths and limitations of this study:**

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

INTRODUCTION

Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes involuntary, rapid and repetitive movement of eyes. Signs of IN usually appear at birth or develop within the first six months of life. The incidence of all forms of IN 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 becomes worse when an affected person stares at an object or is feeling anxious. The cerebellum is thought to have a key role in ocular motor control.² Patients' eye oscillations can be horizontal, vertical, torsional, or combination of the three, and horizontal is most common. To date, the pathogenesis of IN remains unclear. Many researchers attribute the disease to the abnormal control of the part of brain that is in charge of ocular motor.³ Currently, there is no effective treatment for IN, and 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 IN, IN can be complicated by other diseases like albinism, achromatopsia, Leber congenital amaurosis and visual deprivation at early age. It presents as a component of other neurological syndromes and neurologic diseases.⁶

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

88 retina and in parts of the brain that coordinate eye movement, like the cerebellum and
89 the lateral ventricles.⁹ Although the exact function of this gene is still unclear,
90 previous research showed that *FRMD7* gene mutations may lead to nystagmus by
91 disrupting the normal development of certain nerve cells in the brain and the
92 retina. Most studies focused on its N-terminal FERM domain which may play a role
93 between the plasma and actin cytoskeleton. Previous studies also suggested a link
94 between membrane extension during neuronal development and remodeling of the
95 actin cytoskeleton. The other disease-causing gene of XLIN, *GPR143*, encodes a
96 protein that binds to heterotrimeric G proteins and affects pigment production in the
97 iris, retinal pigment epithelium and skin.¹⁰ This protein is thought to be involved in
98 intracellular signal transduction. Mutations in *GPR143* have also been shown to cause
99 X-Linked ocular albinism type 1(OA1), which is a multi-symptom syndrome that
100 could lead to reduced visual acuity, nystagmus, and strabismus.¹¹ However, a deletion
101 mutation of *GPR143* gene has also been reported in a Chinese IN family without
102 typical phenotype of ocular albinism.¹² Nearly half of X-Linked IN families have
103 genetic defects in the *FRMD7* gene. However, very limited studies investigated the
104 genetic basis of sporadic cases with IN.¹³

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

110 MATERIALS AND METHODS

111 Study subjects

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2
3 112 This study received the approval of The Eye Hospital of Wenzhou Medical
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5 113 University and complied with the Declaration of Helsinki. Written informed consents
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7 114 were obtained from each patient. Twenty Chinese families with idiopathic IN were
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9 115 recruited and peripheral blood samples were collected from some participants. We
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11 116 performed detailed ophthalmologic examinations on selected patients and also tested
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13 117 for visual acuities, slit lamp, intraocular pressure, fundoscopy and angle of head turn.
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119 **DNA extraction**

120 We selected all the twenty probands and some healthy family members from each
121 family and extracted DNA from them using a DNA extraction kit (TIANGEN, Beijing,
122 China) according to the manufacturer's instructions. DNA was quantified using
123 Nanodrop 2000 (Thermal Fisher Scientific, DE, USA).
124

125 **Mutation screening and data analysis**

126 The primers of all coding exons and exon-intron junctions of *FRMD7* and *GPR143*
127 were designed according to the previous studies.^{14,15} Polymerase chain reaction (PCR)
128 and Sanger sequencing were utilized to amplify each exon of *FRMD7* and *GPR143* in
129 each participant. Sequencing results were analyzed by Mutation Surveyor
130 (Softgenetics, PA) and the potential pathogenic effects of the mutations on protein
131 function were estimated using MutationTaster (<http://www.mutationtaster.org>),
132 Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>) and SIFT
133 (http://sift.jcvi.org/www/SIFT_enst_submit.html). The annotation of frequency was
134 set according to the 1000 Genomes (<http://www.1000genomes.org/>). The co-
135 segregation of putative causative variants was performed in the family members.
136

137 **RESULTS**

138 **Clinical observations**

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

148 **Mutation detection**

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

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3 187 X-Linked IN families are shown in Figure S1. However, none of putative mutations
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5 188 were identified in *FRMD7* or *GPR143* in any sporadic cases, suggesting very limited
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7 189 etiology contribution of *FRMD7* or *GPR143* in the simplex IN patients.
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9
10 190 Taken together, we screened *FRMD7* and *GPR143* genes in fifteen patients with
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12 191 simplex IN and five probands with XLIN. Finally, two disease-causing mutations
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14 192 were identified in *FRMD7* gene in two XLIN families, with a detection rate of 40%.
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16 193 However, none candidate variants were discovered in any simplex IN patients. The
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18 194 results demonstrated that the mutations in *FRMD7* appeared to be the major genetic
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20 195 cause of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our
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22 196 findings provide further insights into the mutation spectrum of *FRMD7*, which could
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24 197 be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus
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26 198 patients.
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203

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.

208

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214

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216

Patient consent: Obtained.

218

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 F1 and 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.

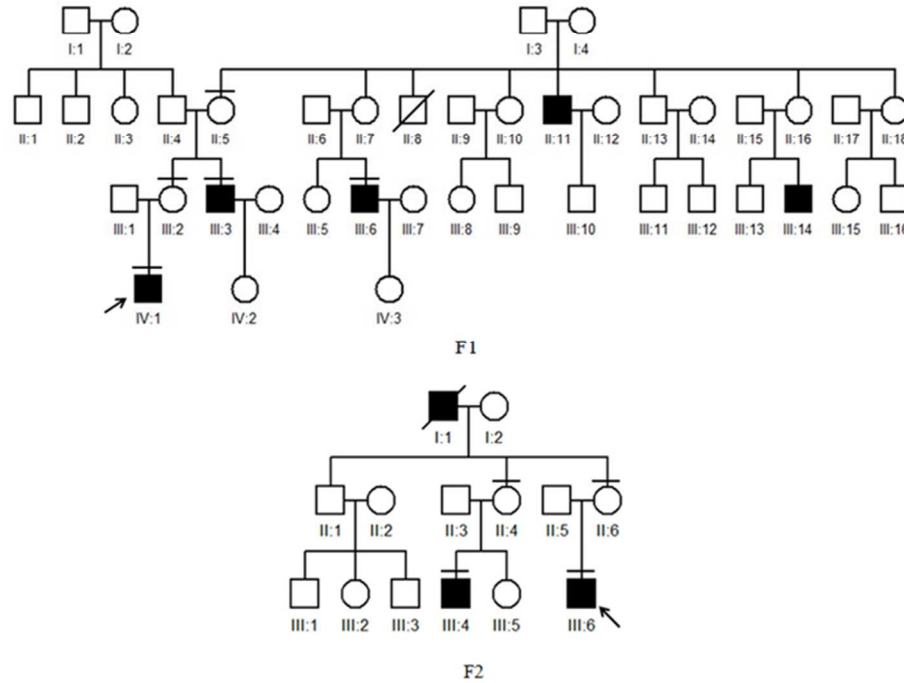


Figure 1. Pedigrees of two recruited families with X-Linked congenital nystagmus.
67x55mm (300 x 300 DPI)

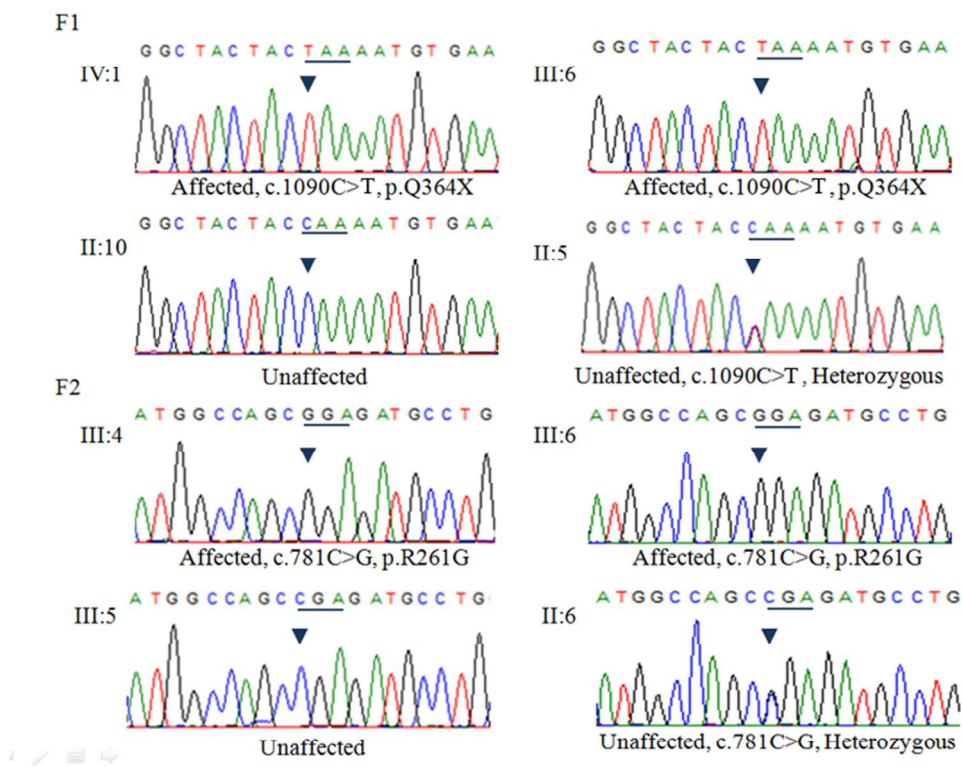


Figure 2. DNA sequence chromatograms of the unaffected and affected members in family F1 and F2. 67x58mm (300 x 300 DPI)



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

