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

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

27	Objectives: Infantile nystagmus (IN) is a genetically heterogeneous condition
28	characterized by involuntary rhythmic oscillations of the eyes accompanied with
29	vision impairment of different severity. Two genes have been identified as major
30	disease-causing genes for IN, FRMD7 and GPR143. The aim of our study is to detect
31	the genetic basis of both sporadic IN and X-Linked IN.
32	Design: Prospective analysis.
33	Patients: Twenty Chinese patients underwent molecular genetic analysis, including
34	fifteen sporadic IN cases and five X-Linked IN families, was recruited in this study.
35	We first performed PCR-based DNA direct sequencing of the entire coding region
36	and splice junctions of the above two genes in the recruited individuals from the two
37	pedigrees. Then mutational analysis and co-segregation confirmation were performed.
38	Setting: All clinical examinations and genetic experiments were performed in the Eye
20	Hospital of Wenzhou Medical University
39	Hospital of Weizhou Wedreal Oniversity.
39 40	Results: Two mutations in <i>FRMD7</i> gene, including one novel nonsense mutation
40 41	Results: Two mutations in <i>FRMD7</i> gene, including one novel nonsense mutation (c.1090C>A, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G),
39 40 41 42	Results: Two mutations in <i>FRMD7</i> 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).
 39 40 41 42 43 	Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any
 39 40 41 42 43 44 	Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases.
 39 40 41 42 43 44 45 	 Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases. Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> appeared
 39 40 41 42 43 44 45 46 	 Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases. Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> appeared to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our
 39 40 41 42 43 44 45 46 47 	 Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases. Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> 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 <i>FRMD7</i>, which could
 39 40 41 42 43 44 45 46 47 48 	 Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases. Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> 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 <i>FRMD7</i>, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus
 39 40 41 42 43 44 45 46 47 48 49 	 Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases. Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> 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 <i>FRMD7</i>, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.

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51	Strengths and limitations of this study:
52	• As very few studies focused on the sporadic cases, the aim of our study is 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 suggested 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 the future study.
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62	INTRODUCTION
63	Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes
64	involuntary, rapid and repetitive movements of the eyes. Signs of IN usually appear at
65	birth or develop within the first six months of life. The incidence of all forms of
66	infantile nystagmus is estimated to be 14 per 10,000 individuals. ¹ The most common
67	symptom of IN is visual acuity impairment, which is caused by excessive motion of
68	images on the retina or by the movement of images away from the fovea. The
69	abnormal eye movement sometimes become worsen when an affected person stares at
70	an object or is feeling anxious. Patients usually tend to tilt their head in order to
71	compensate for the abnormal eye movement. ² Patients' eye oscillations can be
72	horizontal, vertical, torsional, or combination of the three, and horizontal is the most
73	common. To date, the pathogenesis of IN remains unclear. Many researchers attribute
74	the disease to the abnormal control of the part of the brain that is in charge of ocular
75	motor. ³ Currently, there is no effective treatment for IN, only very limited surgical,

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76	optical or pharmaceutical therapies have been employed to improve nystagmus
77	waveforms and vision acuity. ^{4,5} With the exception of idiopathic infantile nystagmus,
78	IN can be complicated by other diseases like albinism, achromatopsia, Leber
79	congenital amaurosis and visual deprivation at early age. It present as a component of
80	other neurological syndromes and neurologic diseases. ⁶
81	Infantile nystagmus can be inherited as an X-Linked, an autosomal recessive or an
82	autosomal dominant disease with incomplete penetrance and variable expressivity.
83	Although the inheritance pattern is heterogeneous, the most common form of IN
84	follows that of an X-Linked pattern. To date, two major genes have been defined as
85	the causative genes of hereditary X-Linked infantile nystagmus (XLIN). ⁷ The FRMD7
86	gene contains an N-terminal FERM domain with high conservation and FERM-
87	adjacent domain without significant homology. Mutations in FRMD7 gene is
88	currently the most common cause of XLIN. It is mainly expressed in the retina and in
89	parts of the brain that coordinate eye movement, like the cerebellum and the lateral
90	ventricles. ⁸ Although the exact function of this gene is still unclear, previous research
91	suggests that FRMD7 gene mutations may lead to nystagmus by disrupting the normal
92	development of certain nerve cells in the brain and the retina. Most studies focus on
93	its N-terminal FERM domain which may play a role between the plasma and actin
94	cytoskeleton. Previous studies also suggest a link between membrane extension
95	during neuronal development and remodeling of the actin cytoskeleton. ⁹ The other
96	disease-causing gene of XLIN, GPR143, encodes a protein that binds to
97	heterotrimeric G proteins and affects pigment production in the iris, retinal pigment
98	epithelium and skin. ¹⁰ This protein is thought to be involved in intracellular signal
99	transduction. Mutations in GPR143 have been shown to cause X-Linked ocular
100	albinism type 1(OA1), which is a multi-symptom syndrome that could lead to reduced

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101	visual acuity, nystagmus, strabismus and so on. ^{11,12} However, a deletion mutation of
102	GPR143 gene has also been reported in a Chinese IN family without typical
103	phenotype of ocular albinism. ¹³ Nearly half of X-Linked IN families have genetic
104	defects in the FRMD7 gene. However, very limited studies investigate the genetic
105	basis of sporadic cases with IN.
106	In this study, we recruited a Chinese cohort of IN patients including 15 sporadic
107	cases and 5 families with X-Linked IN. We performed molecular genetic analysis on
108	FRMD7 and GPR143 in participants from these 20 unrelated patients to detect the
109	causative mutations.
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111	MATERIALS AND METHODS
112	Study subjects
113	This study received the approval of The Eye Hospital of Wenzhou Medical
114	University and complied with the Declaration of Helsinki. Written informed consents
115	were obtained from each patient. Twenty Chinese families exhibited X-Linked IN
116	were recruited and peripheral blood samples were collected from some participants.
117	We performed detailed ophthalmologic examinations on selected patients and also
118	tested for visual acuities, slit lamp, intraocular pressure, fundoscopy and angle of head
119	turn.
120	
121	DNA extraction
122	We selected all the twenty probands and some healthy family members from each
123	family and extracted DNA from them using a DNA Extraction Kit (TIANGEN,
124	Beijing) according to the manufacturer's instructions. DNA was quantified using
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.
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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
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151	candidate mutations were identified. A novel missense mutation, c.1090C>A
152	(p.Q364X), in exon 12 of <i>FRMD7</i> , 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 <i>FRMD7</i> , 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 GPR143 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

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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

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176	of identifying disease-causing genetic defects in IN population, we recruited twenty
177	infantile nystagmus families, including fifteen sporadic cases and five X-Linked IN
178	families. Since FRMD7and GPR143 have been previously identified as the pathogenic
179	genes of X-Linked IN, we focused on screening exons and exon/intron boundaries of
180	the two genes using Sanger sequencing. ¹⁶ Up to now, more than 40 mutations in the
181	FRMD7 gene have been found which mainly concentrated in two key regions
182	including FERM and FA domains. ¹⁷ However, the function of the protein still remains
183	poorly understood. Previous studies demonstrated the role of <i>FRMD7</i> in the
184	regulation of neuronal cytoskeletal dynamics at the growth cone through Rho GTPase
185	signaling. Genetic defects in FRMD7 may damage the recruitment and the activation
186	of the Rho family of GTPases (Cdc42, Rac1, and RhoA) and their regulators. ^{18,19} Two
187	mutations in FRMD7 gene, including one novel nonsense mutation (c.1090C>A,
188	p.Q364X) and one reported missense mutation (c.781C>G, p.R261G), were identified
189	in this study. In family F1, the affected males harbored the novel nonsense mutation
190	(c.1090C>A, p.Q364X) and all exhibited typical manifestations of IN. Because the
191	females in family F1 that carried a heterozygous mutation showed no disease
192	phenotypes, we conclude that the penetrance of the mutation in this family was 100%,
193	which was consistent with IN diagnosis of the offsprings. Similar to that in family F1,
194	the mutation (p.R261G) identified in family F2 was also recessive and has previously
195	been reported in another Chinese X-Linked IN family. Taken together, we identified
196	disease-causing mutations in two X-Linked IN families, with the detection rate of 40%
197	(2/5). However, none of putative mutations were identified in <i>FRMD7</i> or <i>GPR143</i> in
198	any sporadic cases, suggesting the very limited etiology contribution of FRMD7 or
199	GPR143 in the simplex IN patients.

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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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210	Acknowledgements: We thank the patients and their family members for their
211	participation in this study.
212	
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.
217	
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222	sciences (WKJ-ZJ-1417 to Z.B.J.).
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224	Competing interests: None.
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226	Patient consent: Obtained.				
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228	Ethics	approval: This study was conducted with the approval of the Eye Hospital of			
229	Wenzł	nou Medical University.			
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231	Prove	nance and peer review: Not commissioned; externally peer reviewed.			
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233	Data s	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	- 0	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

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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 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)

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



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

Section/Topic	ltem #	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/	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe	5,6
measurement		comparability of assessment methods if there is more than one group	
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			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed	6,7		
		eligible, included in the study, completing follow-up, and analysed			
		(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	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	7-9		
		similar studies, and other relevant evidence			
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	9		
		which the present article is based			

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

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# SCHOLARONE[™] Manuscripts

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1	Molecular genetic analysis of patients with sporadic and X-Linked infantile
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4	Hui Chen, ^{1,2} Xiu-Feng Huang, ^{1,2} Huan-Yun Yu, ¹ Dong-Jun Xing, ¹ Liang Ye, ¹ Su-
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# 26 ABSTRACT

27	Objectives: Infantile nystagmus (IN) is a genetically heterogeneous condition
28	characterized by involuntary rhythmic oscillations of the eyes accompanied with
29	vision impairment of different severity. Two genes have been identified as major
30	disease-causing genes for IN, FRMD7 and GPR143. The aim of our study is to detect
31	the genetic basis of both sporadic IN and X-Linked IN.
32	Design: Prospective analysis.
33	Patients: Twenty Chinese patients underwent molecular genetic analysis, including
34	fifteen sporadic IN cases and five X-Linked IN families, was recruited in this study.
35	We first performed PCR-based DNA direct sequencing of the entire coding region
36	and splice junctions of the above two genes in the recruited individuals from the two
37	pedigrees. Then mutational analysis and co-segregation confirmation were performed.
38	Setting: All clinical examinations and genetic experiments were performed in the Eye
39	Hospital of Wenzhou Medical University.
40	Results: Two mutations in FRMD7 gene, including one novel nonsense mutation
40 41	<b>Results:</b> Two mutations in <i>FRMD7</i> gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G),
40 41 42	<b>Results:</b> Two mutations in <i>FRMD7</i> 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).
40 41 42 43	<b>Results:</b> Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any
40 41 42 43 44	Results: Two mutations in <i>FRMD7</i> 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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases.
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40 41 42 43 44 45 46	<ul> <li>Results: Two mutations in <i>FRMD7</i> gene, including one novel nonsense mutation</li> <li>(c.1090C&gt;T, p.Q364X) and one reported missense mutation (c.781C&gt;G, p.R261G),</li> <li>were identified in two X-Linked IN families, with the detection rate of 40% (2/5).</li> <li>However, none of putative mutations were identified in <i>FRMD7</i> or <i>GPR143</i> in any</li> <li>sporadic cases.</li> <li>Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> appeared</li> <li>to be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our</li> </ul>
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40 41 42 43 44 45 46 47 48 49	<ul> <li>Results: Two mutations in <i>FRMD7</i> gene, including one novel nonsense mutation (c.1090C&gt;T, p.Q364X) and one reported missense mutation (c.781C&gt;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 <i>FRMD7</i> or <i>GPR143</i> in any sporadic cases.</li> <li>Conclusions: In conclusion, the results suggested that mutations in <i>FRMD7</i> 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 <i>FRMD7</i>, which could be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus patients.</li> </ul>

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51	Strengths and limitations of this study:
52	• As very few studies focused on the sporadic cases, the aim of our study is 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 suggested 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 the future study.
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62	INTRODUCTION
63	Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes
64	involuntary, rapid and repetitive movements of the eyes. Signs of IN usually appear at
65	birth or develop within the first six months of life. The incidence of all forms of
66	infantile nystagmus is estimated to be 14 per 10,000 individuals. ¹ The most common
67	manifestation of IN is visual acuity impairment, which is caused by excessive motion
68	of images on the retina or by the movement of images away from the fovea. The
69	abnormal eye movement sometimes become worse when an affected person stares at
70	an object or is feeling anxious. The cerebellum is thought to have a key role to ocular
71	motor control. ² Patients' eye oscillations can be horizontal, vertical, torsional, or
72	combination of the three, and horizontal is the most common. To date, the
73	pathogenesis of IN remains unclear. Many researchers attribute the disease to the
74	abnormal control of the part of the brain that is in charge of ocular motor. ³ Currently,
75	there is no effective treatment for IN, only very limited surgical, optical or

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76	pharmaceutical therapies have been employed to improve nystagmus waveforms and
77	visual acuity. ^{4,5} With the exception of idiopathic infantile nystagmus, IN can be
78	complicated by other diseases like albinism, achromatopsia, Leber congenital
79	amaurosis and visual deprivation at early age. It present as a component of other
80	neurological syndromes and neurologic diseases. ⁶
81	Infantile nystagmus displayed extreme genetic and clinical heterogeneity, and the
82	most common form of IN follows that of an X-Linked pattern. To date, two major
83	genes have been defined as the causative genes of hereditary X-Linked infantile
84	nystagmus (XLIN). ⁷ The <i>FRMD7</i> gene contains an N-terminal FERM domain with
85	high conservation and FERM-adjacent domain without significant homology.
86	Mutations in FRMD7 gene is currently the most common cause of XLIN. ⁸ It is mainly
87	expressed in the retina and in parts of the brain that coordinate eye movement, like the
88	cerebellum and the lateral ventricles. ⁹ Although the exact function of this gene is still
89	unclear, previous research suggests that FRMD7 gene mutations may lead to
90	nystagmus by disrupting the normal development of certain nerve cells in the brain
91	and the retina. Most studies focus on its N-terminal FERM domain which may play a
92	role between the plasma and actin cytoskeleton. Previous studies also suggest a link
93	between membrane extension during neuronal development and remodeling of the
94	actin cytoskeleton. The other disease-causing gene of XLIN, GPR143, encodes a
95	protein that binds to heterotrimeric G proteins and affects pigment production in the
96	iris, retinal pigment epithelium and skin. ¹⁰ This protein is thought to be involved in
97	intracellular signal transduction. Mutations in GPR143 have also been shown to cause
98	X-Linked ocular albinism type 1(OA1), which is a multi-symptom syndrome that
99	could lead to reduced visual acuity, nystagmus, strabismus and so on. ¹¹ However, a
100	deletion mutation of GPR143 gene has also been reported in a Chinese IN family

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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.
108	
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.
117	
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).
123	
124	Mutation screening and data analysis
	5

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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.
135	
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.
145	
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

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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, FRMD7and 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}
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151	families with XLIN (Table 1). A novel nonsense mutation, c.1090C>T (p.Q364X),
152	exon 12 of FRMD7, which would lead to a stop codon at position 364, was detected
153	by Sanger sequencing analysis of a patient (IV:1) from family F1 (Figure 1).
154	Sequencing analysis of a patient (III:4) from family F2 (Figure 1) showed that he
155	harbored a missense mutation (c.781C>G, p.R261G) in exon 10 of FRMD7, which
156	would result in amino acid substitution of arginine by glutamate at the position of 2
157	(Figure 2). All protein prediction programs assigned this mutation as damaging and
158	impactful of protein function. Notably, this mutation has previously been reported b
159	other researchers. ¹⁶ This mutation also followed an X-Linked recessive inheritance.
160	Both of mutations Q364X and R261G were located in highly conserved regions
161	(Figure 3). There are five affected males in family F1 and two affected males in
162	family F2. The age distribution of affected individuals ranged from 4 to 35 years an
163	the age of onset was between 3 and 6 months of age (Table 2). Of note, none of the
164	participants showed abnormalities in GPR143 gene. Taken together, we conclude the
165	the two variants, c.1090C>T (p.Q364X) and c.781C>G (p.R261G) in FRDM7 gene,
166	are the disease-causing mutations in the two Chinese families with XLIN.
167	
168	DISCUSSION
169	The genetic etiology of IN is not yet fully understood, especially the sporadic
170	cases. ¹⁷ In this study, we recruited twenty infantile nystagmus families, including
171	fifteen sporadic cases and five X-Linked IN families. The major disease-causing
172	genes, FRMD7and GPR143 have been screened.

Up to now, more than 40 mutations in the FRMD7 gene has

mainly concentrated in two key regions including FERM and

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175	However, the function of the protein still remains poorly understood. Previous studies
176	demonstrated the role of FRMD7 in the regulation of neuronal cytoskeletal dynamics
177	at the growth cone through Rho GTPase signaling. ^{20,21} Two mutations in <i>FRMD7</i>
178	gene, including one novel nonsense mutation (c.1090C>T, p.Q364X) and one
179	reported missense mutation (c.781C>G, p.R261G), were identified in this study. In
180	family F1, the affected males harbored the novel nonsense mutation (c.1090C>T,
181	p.Q364X) and all exhibited typical manifestations of IN. The mutation (p.R261G)
182	identified in family F2 was also recessive and has previously been reported in another
183	Chinese X-Linked IN family. Taken together, we identified disease-causing mutations
184	in two X-Linked IN families, with the detection rate of 40% (2/5). The pedigrees of
185	other three X-Linked IN families were displayed in the Figure S1. However, none of
186	putative mutations were identified in FRMD7 or GPR143 in any sporadic cases,
187	suggesting the very limited etiology contribution of FRMD7 or GPR143 in the
188	simplex IN patients.
189	Taken together, we screened FRMD7 and GPR143 genes in fifteen patients with
190	simplex IN and five probands with XLIN. Finally, two disease-causing mutations
191	were identified in <i>FRMD7</i> gene in two XLIN families, with the detection rate of 40%.
192	However, none candidate variants were discovered in any simplex IN patients. The
193	results demonstrated that mutations in FRMD7 appeared to be the major genetic cause
194	of hereditary X-Linked nystagmus, but not of sporadic nystagmus patients. Our
195	findings provide further insights into the mutation spectrum of FRMD7, which could
196	be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus
197	patients.

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201	
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203	collected the samples and performed the experiments, XFH and FZ performed data
204	analyses, XFH and ZBJ wrote the manuscript. All authors have read and approved the
205	final manuscript.
206	
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214	
215	Patient consent: Obtained.
216	
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219	
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221	
222	Data sharing statement: No additional data available.
223	
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# Table 1. The summary of variants.

Family	Proband	Gene	Variant	Туре	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
				13	3	
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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	- 0	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 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)





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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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1	molecular genetic analysis of patients with sporadic and A-Linked infantile
2	nystagmus
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4	Hui Zhao, ² Xiu-Feng Huang, ¹ Huan-Yun Yu, ¹ Wen-Li Deng, ¹ Xin-Lan Lei, ¹ Dong-
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# 26 ABSTRACT

27	Objectives: Infantile nystagmus (IN) is a genetically heterogeneous condition
28	characterized by involuntary rhythmic oscillations of eyes accompanied with vision
29	impairment of different severity. Two genes have been identified as major disease-
30	causing genes for IN, FRMD7 and GPR143. The aim of our study was to detect the
31	genetic basis of both sporadic IN and X-Linked IN.
32	Design: Prospective analysis.
33	Patients: Twenty Chinese patients underwent molecular genetic analysis, including
34	fifteen sporadic IN cases and five X-Linked IN families, were recruited in this study.
35	We first performed PCR-based DNA sequencing of the entire coding region and the
36	splice junctions of the two genes mentioned above in the recruited individuals from
37	the two pedigrees. Then mutational analysis and co-segregation confirmation were
38	performed.
39	Setting: All clinical examinations and genetic experiments were performed in the Eye
40	Hospital of Wenzhou Medical University.
41	Results: Two mutations in FRMD7 gene, including one novel nonsense mutation
42	(c.1090C>T, p.Q364X) and one reported missense mutation (c.781C>G, p.R261G),
43	were identified in two X-Linked IN families, with a detection rate of 40% (2/5).
44	However, none of putative mutations were identified in FRMD7 or GPR143 in any
45	sporadic cases.
46	Conclusions: In summary, the results suggest that mutations in FRMD7 appeared to
47	be the major genetic cause of X-Linked IN, but not of sporadic IN patients. Our
48	findings provide further insights into the mutation spectrum of FRMD7, which could
49	be helpful for future genetic diagnosis and genetic counseling in Chinese nystagmus
50	patients.

More samples are required

1		
2 3 4	51	Strengths and limitations of this study:
5	52	• As very few studies focused on the sporadic cases, the aim of our study was to
7 8	53	detect the genetic basis of both sporadic IN and X-Linked IN.
9 10	54	Two mutations in FRMD7 gene, including one novel nonsense mutation, were
11 12	55	identified in two X-Linked IN families.
13 14 15	56	The results suggest that mutations in FRMD7 appeared to be the major genetic
16 17	57	cause of X-Linked IN, but not of sporadic IN patients. More samples are require
18 19	58	in future study.
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## 63 INTRODUCTION

64	Infantile nystagmus (IN), as the most common oculomotor disorder, usually causes
65	involuntary, rapid and repetitive movement of eyes. Signs of IN usually appear at
66	birth or develop within the first six months of life. The incidence of all forms of IN is
67	estimated to be 14 per 10,000 individuals. ¹ The most common manifestation of IN is
68	visual acuity impairment, which is caused by excessive motion of images on the retina
69	or by the movement of images away from the fovea. The abnormal eye movement
70	sometimes becomes worse when an affected person stares at an object or is feeling
71	anxious. The cerebellum is thought to have a key role in ocular motor control. ²
72	Patients' eye oscillations can be horizontal, vertical, torsional, or combination of the
73	three, and horizontal is most common. To date, the pathogenesis of IN remains
74	unclear. Many researchers attribute the disease to the abnormal control of the part of
75	brain that is in charge of ocular motor. ³ Currently, there is no effective treatment for
76	IN, and only very limited surgical, optical or pharmaceutical therapies have been
77	employed to improve nystagmus waveforms and visual acuity. ^{4,5} With the exception
78	of idiopathic IN, IN can be complicated by other diseases like albinism,
79	achromatopsia, Leber congenital amaurosis and visual deprivation at early age. It
80	presents as a component of other neurological syndromes and neurologic diseases. ⁶
81	IN displays extreme genetic and clinical heterogeneity, and the most common form
82	of IN follows an X-Linked pattern. To date, two major genes FRMD7 and GPR143
83	have been identified as the causative genes of hereditary X-Linked infantile
84	nystagmus (XLIN). ⁷ The FRMD7 gene contains an N-terminal FERM (F for 4.1
85	protein, E for ezrin, R for radixin and M for moesin) domain with high conservation
86	and a FERM-adjacent domain without significant homology. Mutations in FRMD7
87	gene are currently the most common cause of XLIN. ⁸ It is mainly expressed in the

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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.
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- 110 MATERIALS AND METHODS
- 111 Study subjects

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112	This study received the approval of The Eye Hospital of Wenzhou Medical
113	University and complied with the Declaration of Helsinki. Written informed consents
114	were obtained from each patient. Twenty Chinese families with idiopathic IN were
115	recruited and peripheral blood samples were collected from some participants. We
116	performed detailed ophthalmologic examinations on selected patients and also tested
117	for visual acuities, slit lamp, intraocular pressure, fundoscopy and angle of head turn.
118	
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 <i>FRMD7</i> and <i>GPR143</i>
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-
- segregation of putative causative variants was performed in the family members.

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.
147	
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.

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162	Both of mutations Q364X and R261G were located in highly conserved regions
163	(Figure 3). There are five affected males in family F1 and two affected males in
164	family F2. The age distribution of the affected individuals ranged from 4 to 35 years
165	and the age of onset was between 3 and 6 months of age (Table 2). Of note, none of
166	the participants showed abnormalities in GPR143 gene. Taken together, we conclude
167	that the two variants, c.1090C>T (p.Q364X) and c.781C>G (p.R261G) in FRDM7
168	gene, are the disease-causing mutations in the two Chinese families with XLIN.
169	
170	DISCUSSION
171	The genetic etiology of IN is not yet fully understood, especially the sporadic
172	cases. ¹⁷ In this study, we recruited twenty IN families, including fifteen sporadic cases
173	and five X-Linked IN families. The major disease-causing genes, FRMD7and
174	GPR143 have been screened.
175	Up to now, more than 40 mutations have been found in the FRMD7 gene which
176	mainly concentrated in two key regions: FERM and FA domains. ^{18,19} However, the
177	function of the protein still remains poorly understood. Previous studies demonstrated
178	the role of <i>FRMD7</i> in the regulation of neuronal cytoskeletal dynamics at the growth
179	cone through Rho GTPase signaling. ^{20,21} Two mutations in the <i>FRMD7</i> gene,
180	including one novel nonsense mutation (c.1090C>T, p.Q364X) and one reported
181	missense mutation (c.781C>G, p.R261G), were identified in this study. In family F1,
182	the affected males harbored the novel nonsense mutation (c.1090C>T, p.Q364X) and
183	all exhibited typical manifestations of IN. The mutation (p.R261G) identified in
184	family F2 was also recessive and has previously been reported in another Chinese X-
185	Linked IN family. Taken together, we identified disease-causing mutations in two X-
186	Linked IN families, with a detection rate of $40\%$ (2/5). The pedigrees of other three

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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 .is a.. patients.

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203	
204	Contributions: ZBJ, HZ and XPY conceived the idea, WLD, XLL, DJX, HYY, LY,
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206	performed data analyses, XFH and ZBJ wrote the manuscript. All authors have read
207	and approved the final manuscript.
208	
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214	
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216	
217	Patient consent: Obtained.
218	
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220	Wenzhou Medical University.
221	
222	Provenance and peer review: Not commissioned; externally peer reviewed.
223	
224	Data sharing statement: No additional data available.
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# Table 1. The summary of variants.

Family	Proband	Gene	Variant	Туре	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
				15	5	

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	- 0	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 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)

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