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Novel mutations of the *FRMD7* gene in X-linked congenital motor nystagmus

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Purpose: Congenital motor nystagmus (CMN) is a relatively common oculomotor disorder characterized by bilateral uncontrollable ocular oscillations. Recently, the *FRMD7* gene mutation has been identified as the genetic cause of CMN. The purpose of this study was to identify mutations of the *FRMD7* gene in Chinese patients with CMN.
Methods: Clinical data and genomic DNA of three Chinese CMN families were collected after informed consent. Genescan by two-point linkage analysis combined with haplotype analysis was performed and mutation screening of the *FRMD7* gene was conducted by direct sequencing.
Results: Maximum two-point LOD scores of 2.00, 1.76, and 1.16 at θ=0.00 were obtained with markers in proximity to the *FRMD7* gene on chromosome Xp26 in the three CMN families. Mutation screening in the *FRMD7* gene identified two novel missense mutations (c.781C>G and c.886G>C) and one reported nonsense mutation (c.1003C>T). These nucleotide alterations were not seen in unaffected members of the families or in 100 unrelated control subjects.
Conclusions: This study widens the mutation spectrum of the *FRMD7* gene.

Congenital motor nystagmus (CMN), also termed idiopathic congenital nystagmus, is a common hereditary disorder characterized by bilateral ocular oscillations that occur in the first 6 months of life [1-3]. The prevalence of CMN in the human population is estimated to be between 1:1,000 and 1:1,500 [4,5]. CMN is distinct from other genetic ocular disorders in which nystagmus accompanies a clinically apparent defect in the visual sensory system [6]. CMN could cause visual impairment and visual acuity can be diminished, usually slightly to moderately. The mechanism of CMN remains unclear and it is presumed to be secondary to an abnormal development of those ocular motor areas of the brain that control fixation [2,7,8]. There is no curative treatment currently for the disease [9,10]. CMN can be inherited in various patterns and X-linked inheritance with incomplete penetrance and variable expressivity is probably the most common form. CMN with X-linked inheritance has been mapped to two regions: Xp11.4-p11.3 [1] and Xq26-q27 [2,11-13]. The mutation in the *FRMD7* gene (Genbank NM_194277) is the major cause of familial X-linked CMN mapped to Xq26-q27 [14,15]. We recently collected three CMN families. To confirm whether the *FRMD7* gene was mutated in these families, we undertook linkage analysis and mutation analysis of the *FRMD7* gene in the three CMN families.

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METHODS

Three Chinese families (families 1, 2, and 3) with CMN were from Zhejiang province, in which there were 50 family members including 11 affected males, 12 affected females, and 27 unaffected individuals (Figure 1). Family 1 was previously reported by us [12]. All patients were clinically diagnosed at the Second Affiliated Hospital of Zhejiang University after detailed ocular-neurological examination [1,8]. The fundus photographs were recorded by a TRC.50EX Retinal camera (Topcon Corp, Tokyo, Japan) and the electroretinograms were recorded on an LKC, UTAS-3000 (LKC Technologies Inc., Gaithersburg, MD). All members were recruited with informed consent and with appropriate local and regional ethics review committee approvals. Blood samples were obtained from these family members and DNA was prepared using standard methods. Control DNAs (n=100) from ethnically matched apparently healthy adults were anonymous.

Genotyping and linkage analysis: Microsatellite markers covering the short and long arm of the X chromosome were tested using fluorescent labeled primers, and all these markers were previously used for fine mapping of X-linked CMN [2,11,12]. Alleles were analyzed by GENESCAN Analysis version 3.0 and GENOTYPER version 2.1 software. Two-point LOD scores were calculated by the MLINK program of the LINKAGE package (version 5.1). Linkage analysis was performed for these families as previously described [16]. The disease was specified to be an X-linked dominant trait with penetrances of 0.9 and 1.00 in females and males, respectively. The allele frequency was assumed to be equal, as well as the recombination frequencies in males and females. We assumed

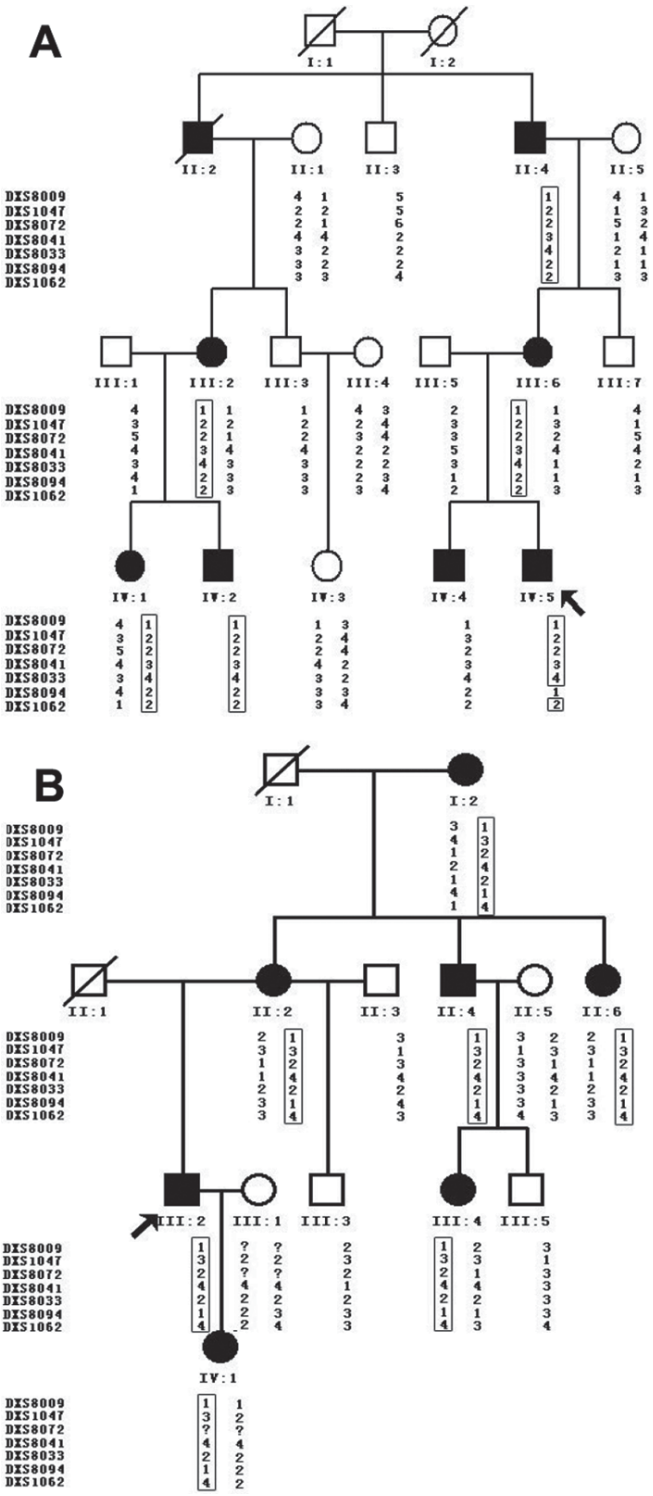

Figure 1. The pedigree of family 2 and family 3. **A**: Haplotype analysis of family 2 showing segregation of five microsatellite markers on the X-chromosome. **B**: Haplotype analysis of family 3 showing segregation of seven microsatellite markers on the X-chromosome. Squares symbolize males, and circles represent females. Black and white symbols denote affected and unaffected status, respectively. Arrows show the probands.

gene frequencies of 0.0001 and no sex difference in recombination.

Sequencing: We designed 17 genomic amplicons to cover the *FRMD7* gene coding region and some flanking intronic sequences in each case. PCR primers were designed by the primer3 online software and the sequences presented in Table 1. For all amplicons, 30 ng genomic DNA was amplified in a volume of 10 µl containing 10X Qiagen HotStar Taq buffer, 1.5 mM $MgSO_4$, 0.2 mM dNTP, 2 µl Q solution, 0.5 µl forward primer, 0.5 µl reverse primer, 4.3 µl ddH_2O and 0.05 U of HotStar Taq. Thermal cycling was performed using a Perkin Elmer Corporator thermal cycler (Applied Biosystems, Foster City, CA). PCR conditions were: 1 cycle of 95 °C for 15 min; 12 cycles of 94 °C for 30 s, 63 °C for 45 s, and 72 °C for 60s; 25 cycles of 94 °C for 30 s, 56 °C for 45 s, and 72 °C for 60 s; and 1 cycle of 72 °C for 10 min.PCR products were

Table 1. Primers for amplification of the *FRMD7* gene

Exon	Sense primers (5'-3')	Antisense primers (5'-3')	Product length (bp)
1	CCTTGGGTGTGCATTACTTC	TTTGCTATTGTTGTCCCTTGAG	459
2	AAACAACACAGAGACAGATAAGTGG	CAATCAGGGAATTGAACCCTAC	385
3	AGGCAGTGGAGCAGTGATTC	GCAGCATGATTTCTTTCATCTC	499
4	CTCGAAGGCAGAGAGGGTAG	CCCTTTGGATGATGAACACC	519
5	GGCACCATTCCTTTCTTGAAT	CAGGCCATGCTGTTTCTCTC	350
6	TTTGGACTGCATTGGCTACA	AGGATCTCAGCGTTTCATGG	353
7	TCATGCACTTTCATCAGAAGC	TGATTGACCATTTCCCTTTC	497
8	TGTGCAAGAGATGGGTCAAG	CTCTGGTTGATTTCTTCAAAGG	368
9	GCTCTGTTTGTGAGCAGTGG	AGGGTGCAATCTTTGATGTG	495
10	AGGTTGTTCTCTGCCTGGTC	GCACTGTCGTTCATGGTACTG	398
11	TGTTTCTCTTGCTCGTGTTGA	TTTTTACACACTGGGATTCTGG	282
12a	CCCTAGAATAGAACATGGATCTTG	TGGGATCAGGGTTAGGATTG	388
12b	CCTTCTTTCACCAATGTGTCC	AATACCAACCTGCTGACCTG	452
12c	CTTTAACACTGAGCCCAATC	TGACTGAGAGCAGGACAAGG	588
12d	ACGGATGTGCCCTATATTCC	GCAACTCCTGCTCTGCAAAC	472
12e	AGCCCAAGGAATATCAGAATG	GCAGTTGGTGTGTTGAAATAAGC	500
12f	AGCCCAAGGAATATCAGAATG	GCAGTTGGTGTGTTGAAATAAGC	500

The primers were selected such that the amplicons contain coding sequence (exons) and the flanking splice junctions. In case of exons 12, nested primers (including 12a, 12b, 12c, 12d, 12e, and 12f) were used for sequencing the amplicons for better fidelity of the data generated.

Table 2. Clinical information of the three families with X-linked congenital motor nystagmus

Family	Affected members	Age of onset	Visual acuity	Strabismus	Electroretinogram	Fundus
1	II:2	3 months	20/40	Orthotropia	Normal	Normal
	II:3	4 months	20/50	Orthotropia	Normal	Normal
	III:1	3 months	20/40	Orthotropia	Normal	Normal
	III:3	5 months	20/80	Orthotropia	Normal	Normal
	III:6	5 months	20/40	Orthotropia	Normal	Normal
	III:8	3 months	20/20	Orthotropia	Normal	Normal
	III:11	6 months	20/100	Orthotropia	Normal	Normal
	IV:3	4 months	20/40	Orthotropia	Normal	Normal
	IV:6	6 months	20/50	NA	NA	NA
2	II:4	5 months	20/50	Orthotropia	Normal	Normal
	III:2	3 months	20/60	NA	NA	NA
	III:6	3 months	20/40	Orthotropia	Normal	Normal
	IV:1	4 months	20/40	Orthotropia	Normal	Normal
	IV:2	4 months	20/60	Orthotropia	Normal	Normal
	IV:4	5 months	20/20	Orthotropia	Normal	Normal
	IV:5	5 months	20/40	Orthotropia	Normal	Normal
3	I:2	4 months	20/200	NA	NA	NA
	II:2	4 months	20/40	Orthotropia	Normal	Normal
	II:4	6 months	20/100	Orthotropia	Normal	Normal
	II:6	5 months	20/40	Orthotropia	Normal	Normal
	III:2	4 months	20/80	Orthotropia	Normal	Normal
	III:4	3 months	20/100	Orthotropia	Normal	Normal
	IV:1	3 months	20/40	Orthotropia	Normal	Normal

This table describes the clinical information of affected members in family 1, family 2, and family 3. "NA" refers to not available. Color vision tests and neurological system examinations were normal and the nystagmus waveform was symmetric horizontal.

digested with Exonuclease I and Shrimp Alkaline Phosphatase to remove the free primers, and both strands were sequenced on an ABI-PRISM 3130 automatic sequencer (Applied Biosystems).

Sequencing results were assembled and analyzed using the SeqMan II program of the Laser gene package (DNA STAR Inc., Madison, WI). For all samples containing an abnormal *FRMD7* amplicon, new PCR products were reamplified from genomic DNA using the same protocols. Mutations identified were confirmed on new independent samples. The sequence variants are numbered according to the GenBank reference cDNA sequence NM_194277 with the "A" of the ATG translation initiation codon being nucleotide 1. To determine the evolutionary conservation of identified substitutions, the ExPASy proteomics server was used to look for homologues of the FRMD7 protein.

RESULTS

In families 1, 2, and 3, an X-linked inheritance pattern was identified. The disease was clearly transmitted from female carriers to affected sons. No male to male transmission was found. The penetrance within these families varied considerably in the female carriers but was consistently complete in the male offsprings. Penetrance was from 54% to about 100% among obligate female carriers (daughters of affected men; family 1, three of three; family 2, two of four; family 3, one of two). The results of the ophthalmologic and neurological system examination were normal. Normal color vision was recorded in all affected individuals. Fundus photograph and electroretinogram examinations were normal in the three individuals tested (family 1, III: 14; family 2, IV: 4; family 3, III: 2; Table 2).

We found no evidence of linkage within the chromosome Xp in Families 1, 2, or 3. However, linkage was initially established without recombination with seven Genethon markers between the regions Xq26-27 closely linked to the *FRMD7* gene. The maximal LOD scores of 2.00, 1.76, and 1.16 were obtained in families 1, 2, and 3 on chromosome Xq26, respectively (Table 3). A recombination event in an affected male in Family 2 refined the location of the CMN gene between markers DXS8009 and DXS8094 (Figure 1).

We identified two novel missense mutations and one reported nonsense mutation in the *FRMD7* gene by direct sequencing of the coding and partial intron regions. In family 1, all affected members had the missense mutation c.781C>G in

Table 3. Linkage results with markers from Xq26-27 in family 2 and family 3

		LOD scores at θ=					
Markers	Family	0.0	0.1	0.2	0.3	0.4	0.5
DXS8009	2	0.56	0.43	0.29	0.15	0.04	0.00
	3	1.16	0.94	0.70	0.44	0.19	0.00
DXS1047	2	-3.06	-0.01	0.1	0.08	0.03	0.00
	3	0.60	0.47	0.32	0.17	0.05	0.00
DXS8072	2	0.56	0.43	0.29	0.15	0.04	0.00
	3	-0.12	0.01	0.03	0.02	0.01	0.00
DXS8041	2	1.76	1.45	1.11	0.74	0.36	0.00
	3	1.16	0.94	0.70	0.44	0.19	0.00
DXS8033	2	1.76	1.45	1.11	0.74	0.36	0.00
	3	0.60	0.47	0.32	0.17	0.05	0.00
DXS8094	2	-3.57	-0.11	0.11	0.16	0.12	0.00
	3	1.16	0.94	0.70	0.44	0.19	0.00
DXS1062	2	1.76	1.45	1.11	0.74	0.36	0.00
	3	1.16	0.94	0.70	0.44	0.19	0.00

Two-point LOD score with markers from Xq26-27 in family 2 and family 3. The maximum two-point LOD score (1.76 and 1.16, respectively) was achieved at DXS8041 at θ=0.

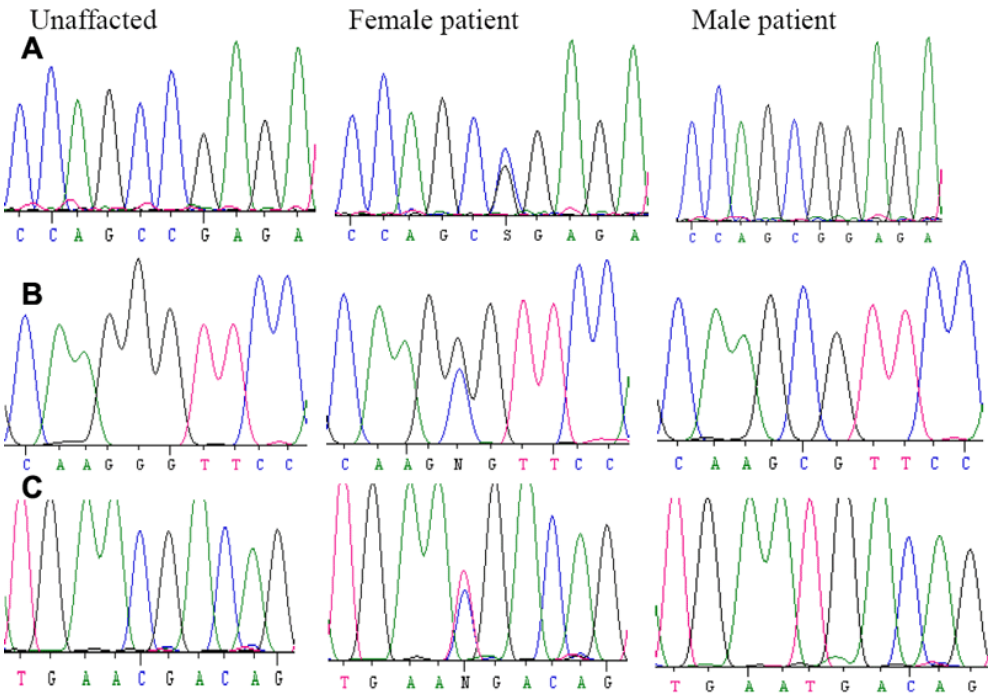

Figure 2. DNA sequence chromatograms of the unaffected and affected members in families 1, 2, and 3. **A**: A single transition was observed at position 781 (C>G) of the *FRMD7* gene, causing a substitution of Arg to Gly at codon 261 (A261G). **B**: A single transition was observed at position 886 (G>C) of the *FRMD7* gene, causing a substitution of Gly to Arg at codon 296 (G296A). **C**: A single transition was observed at position 1003 (C>T) of the *FRMD7* gene, causing a substitution of Arg to a stop codon at codon 335 (R335X). The mutations in female patients are heterozygous and the mutations in male patients are homozygous.

exon 9 (Figure 2A); in family 2, all the patients and obligate carriers had the missense mutation c.886G>C in exon 9 (Figure 2B); in family 3, a previously reported nonsense mutation c.1003C>T in exon 11 [14] was detected (Figure 2C). Obligate female carriers were heterozygous in these mutations and the affected males were homozygous, consistent with X-linked inheritance. All mutations identified above cosegregated with the disease in the families and were absent in the 100 control subjects. The results of the ExPASy proteomics server indicated that the three mutations were within the IMP dehydrogenase/GMP reductase domain (Figure 3).

DISCUSSION

In this study, three families (Families 1, 2, and 3) had high LOD scores suggesting linkage to chromosome Xq, which contains the *FRMD7* gene identified as the cause of CMN. Three mutations, including two novel missense mutations and one reported nonsense mutation, were identified. The three mutations resulted in amino acid substitutions at p.R261G, p.G296R and p.R335X, respectively. These mutations are highly conserved residues that are invariant in *Mus musculus*, *Macaca mulatta*, *Gallus gallus*, *Rattus norvegicus*, and *Canis familiaris*, suggesting that mutations at these locations are functionally significant to the protein (Figure 3).

The mutations identified in this study widen the mutation spectrum of the *FRMD7* gene and this study supports that mutations in the *FRMD7* gene are the major cause of X-linked congenital motor nystagmus as described by Tarpey et al. [14] The mutations identified scattered in almost all the exons and the splice sites as showed in Figure 4. However, the major

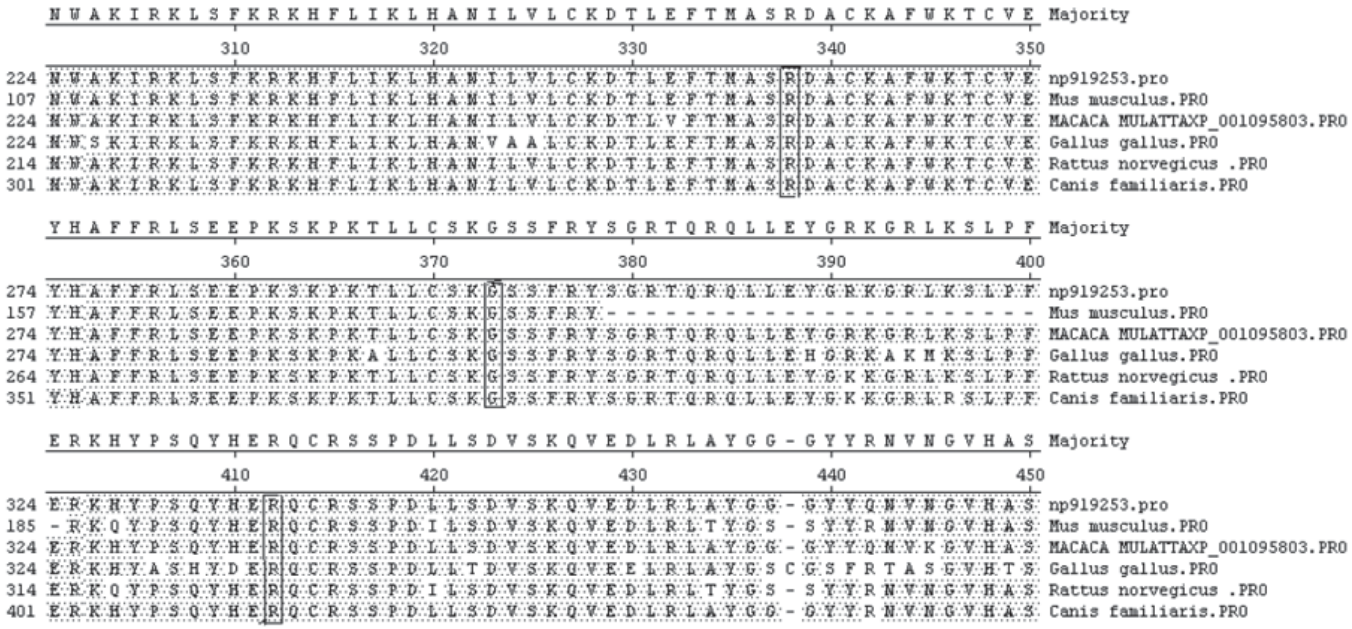

Figure 3. Multiple sequence alignment of FRMD7 proteins and several eukaryotic homologs. For partial analysis the NH_2-terminal and COOH-terminal sequence of PRMD7 are omitted. The lengths of proteins from human (np919253), *Mus musculus*, *Macaca mulatta*, *Gallus gallus*, *Rattus norvegicus*, and *Canis familiaris* are shown. Identity is indicated by gray shading. Amino acids mutated in the various CMN pedigrees are indicated with rectangles above the aligned sequences.

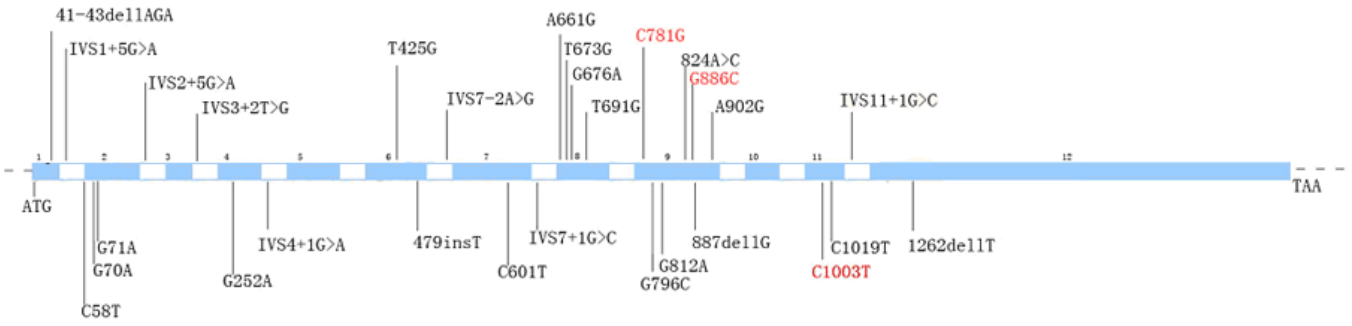

Figure 4. Schematic representation of *FRMD7* cDNA. The mutations identified in this study and previous studies are showed in red and black, respectively.

mutations are clustered at the NH_2-terminus, where homology to B41 and FERM-C domain is present [14]. The COOH-terminus of FRMD7 (amino acid residues 280-714) has no significant homology to other database entries and contains unknown functions. This is thought to be a newly identified member of the FERM family [14]. FERM domains define the band 4.1 superfamily and FERM family members provide a link between the membrane and cytoskeleton and are involved in signal transduction pathways. The B41 protein play structural and regulatory roles in the assembly and stabilization of specialized plasmamembrane domains [17-19]. FERM-C domain is the third structural domain within the FERM domain [17]. In order to predict the possible effects of the various mutations on the protein characteristics, we analyzed the motif of the protein using the ExPASy proteomics server. The results of the ExPASy proteomics assay indicate these mutations occur within the IMP dehydrogenase/GMP reductase domain, which is involved in biosynthesis of guanosine nucleotide [20]. However there is no definitive relationship between the function of IMP dehydrogenase/GMP reductase domain and the mechanism of X-linked CMN. Additional experiments are required to investigate whether a general structure of the protein or specific alterations within possible interacting domains is causing the reduction in function of the various mutated forms of the protein.

The true impact of genetic defects in the *FRMD7* gene and the variable clinical expression of CMN within and between families with the same *FRMD7* gene mutations require further investigation to elucidate the function of this protein. The two novel mutations that we detected in the Chinese population widen the *FRMD7* gene mutation spectrum. It is hoped that the identification of *FRMD7* mutations underlying CMN will enable a more rapid molecular diagnosis and deeper understanding of the pathological mechanisms of CMN.

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