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Identification of Three Novel Mutations in the *FRMD7* Gene for X-linked Idiopathic Congenital Nystagmus

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Idiopathic congenital nystagmus (ICN) consists of involuntary and periodic ocular motility, often with seriously reduced visual acuity. To identify the genetic defects associated with X-linked ICN, we performed PCR-based DNA direct sequencing of two candidate genes, *FRMD7* and *GPR143*, in four families. Mutation analysis led to identification of three novel mutations, p.S260R, p.Q487X, and p.V549Y fsX554, in *FRMD7* in three of the recruited families. Results from structural modeling indicated that the p.S260R may potentially disrupt FRMD7 function through loss of a phosphorylation site and/or interference with protein-protein interactions. Both p.Q487X, and p.V549Y fsX554 mutations were predicted to generate nonfunctional truncated proteins. Using a capture next generation sequencing method, we excluded *CASK* as the responsible gene for the remaining family. Combining sequence analysis and structural modeling, we report three novel mutations in *FRMD7* in three independent families with XLICN, and provide molecular insights for future XLICN diagnosis and treatment.

Idiopathic congenital nystagmus (ICN) causes involuntary and periodic ocular motility, and often seriously reduces visual acuity. It is the most common oculomotor disorder characterized by bilateral uncontrollable ocular oscillation[1], which usually presents in infancy or within the first few months after birth without any other sensory defect[2]. However, the inheritance pattern is heterogeneous, with autosomal dominant, autosomal recessive, or X-linked (XLICN) patterns, among which X-linked is the most common[3,4]. Genes causing XLICN have been mapped at three loci (Xp11.4–p11.3, Xp22, and Xq26–q27)[1,5,6]. XLICN, mapped to Xq26–q27, is linked to the *FRMD7* gene[7], which encodes a member of the protein 4.1 superfamily[7]. *GPR143* resides at Xp22 and mutations within it are primarily linked to ocular albinism (OA), where nystagmus results as a secondary phenotype. However, *GPR143* mutations have been reported in XLICN families, without the classical phenotype of ocular albinism[8–10]. Recently, Watkins et al. reported mutations in the *CASK* gene, locates at Xp11.4–p11.3, is linked with XLICN and mental retardation[11]. Taken together, so far, two genes (*FRMD7* and *GPR143*) have been identified as causative genes for XLICN and *CASK* for XLICN with mental retardation, respectively.

Here, we recruited four families diagnosed with XLICN. Through direct sequencing for the two candidate genes, *FRMD7* and *GPR143*, we identified three novel mutations in *FRMD7* in three independent families. Meanwhile, we have excluded *CASK*, *FRMD7*, and *GPR143* as the disease gene for the remaining family, which provides more evidence to show the genetic heterogeneity associated with XLICN and paves the way for discovering new disease genes for XLICN.

Results

Clinical data. The patterns of inheritance of these four families are X-linked as indicated by the pedigrees (Figure 1). The clinical data are summarized in Table 1. Mental retardation, night blindness, and photophobia are not observed in any of the affected individuals in any family, nor is there any incidence of systemic or other ocular anomalies.

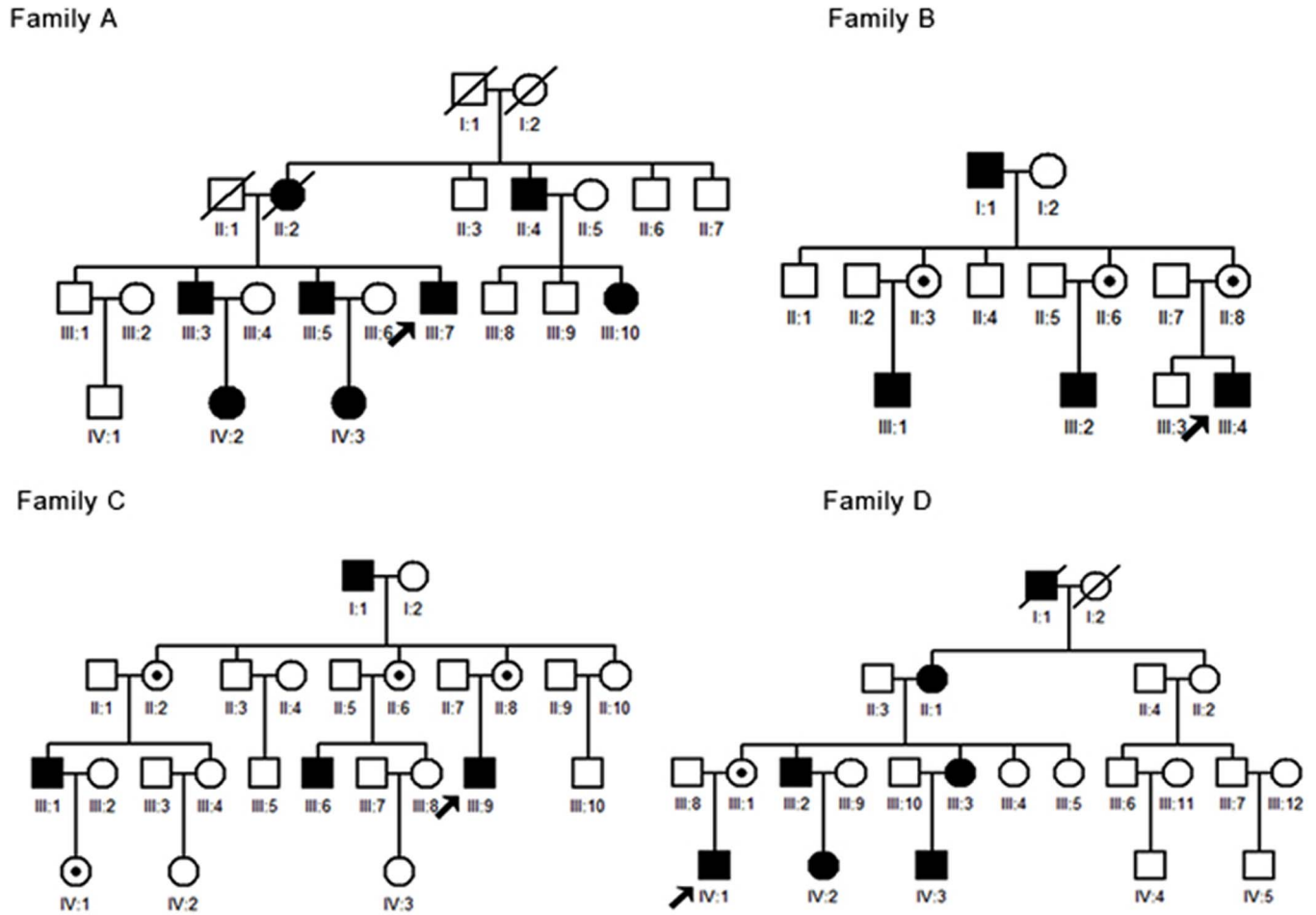

Figure 1 | Pedigrees of four families with X-linked congenital nystagmus. Squares indicate males, circles indicate females, and slashed symbols indicate deceased, black symbols indicate affected individuals, unfilled symbols indicate unaffected individuals, and unaffected, obligate carriers are represented by a dotted circle. Arrow marks the proband.

Mutation identification and analysis. In family A (Figure 1), a novel missense mutation, c.780C > A, was identified at codon 780 (AGC to AGA) in exon 9 of *FRMD7* gene. The c.780C > A mutation causes a substitution of serine to arginine at position 260 (p.S260R) of the FRMD7 protein (Figure 2). The p.S260R mutation occurred at a residue that is evolutionarily highly conserved (Figure 3). Results from online software prediction program strongly suggest that p.S260R variation is a pathogenic mutation. In family B, a nonsense mutation Q487X in exon 12 of *FRMD7* introduces a premature stop codon and produces a truncated protein, which is missing the C-terminal 228 residues (Figure 2, 3). In family C, the deletion mutation (c.1645del G) in exon 12 of *FRMD7*, which is predicted to cause a frame shift at codon 549 and will stop the open reading frame at codon 554, thereby generating a prematurely truncated FRMD7 protein (Figure 2, 3). These mutations cosegregated with affected individuals or carriers in the family (A, B, C), and were not present in any of the unaffected family members (A, B, C) or 200 normal controls. The rest of the coding regions did not show any sequence changes.

We searched these mutations in multiple databases, including the dbSNPs (v130), HapMap, 1000 Genome, and 702 in-house exome data. The candidate mutations were not present in these databases. No mutation in *FRMD7* was identified in family D and no mutations in *GPR143* were detected in any individual among the four families.

We first manually checked of sequencing depth of *CASK* gene. On average, a mean coverage of 88X over the exons in this gene was achieved (Table S1). We further scanned the mutations in *CASK* gene, and no mutations were identified (Table S1).

Table 1 | Clinical findings of individuals in this study

Family	Individual ID	Age (Y)	Age at onset	Best corrected visual acuity at presentation	Clinical findings
Family A	III:5	35	Early Infancy	20/50	Head nodding (+), −4.00
	III:7	24	Early Infancy	20/33	Head nodding (+), −3.00
	IV:3	13	Early Infancy	20/50	Head nodding(+), −3.00
Family B	III:4	22	Early Infancy	20/80	Head nodding (+), −5.50/0.5 × 90
Family C	III:1	27	Early Infancy	20/40	Head nodding (+), −6.00/2 × 90
	III:9	24	Early Infancy	20/40	Head nodding (+), −1.00/3 × 90
Family D	IV:1	25	Early Infancy	20/40	Head nodding (+), −4.00

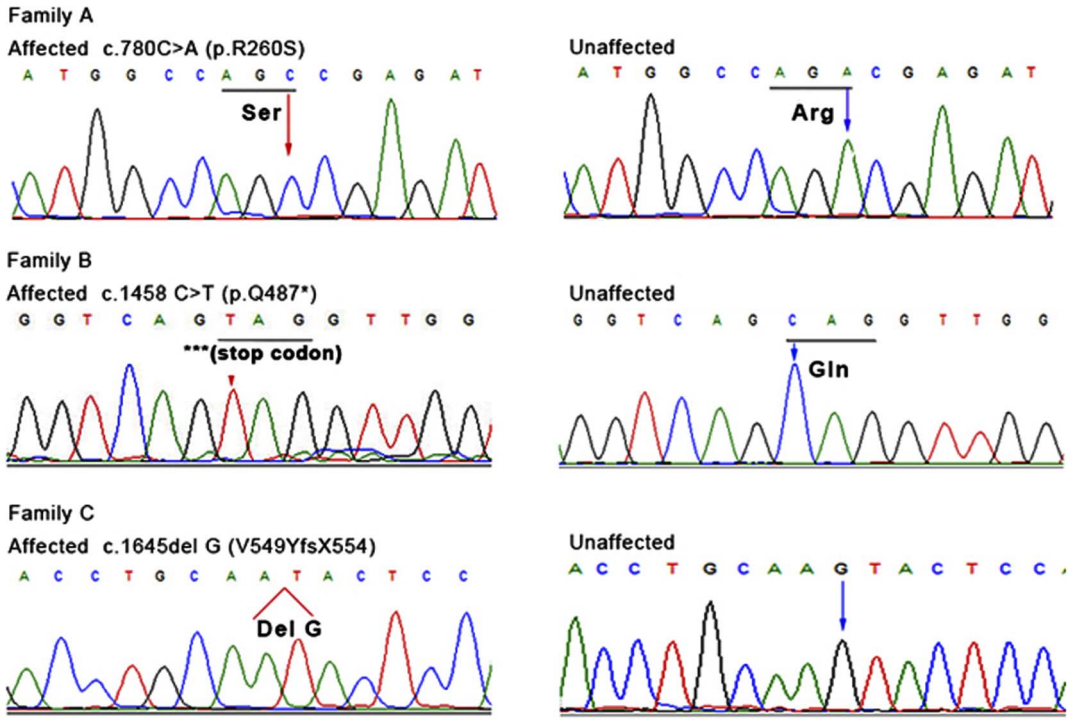

Figure 2 | Sequencing results of affected males and controls. Three mutations of *FRMD7* were identified in three families, which were c.780C > A (p.S260R) in family (A), c.1458 C > T (p.Q487X) in family (B), one G deletion mutation in family (C).

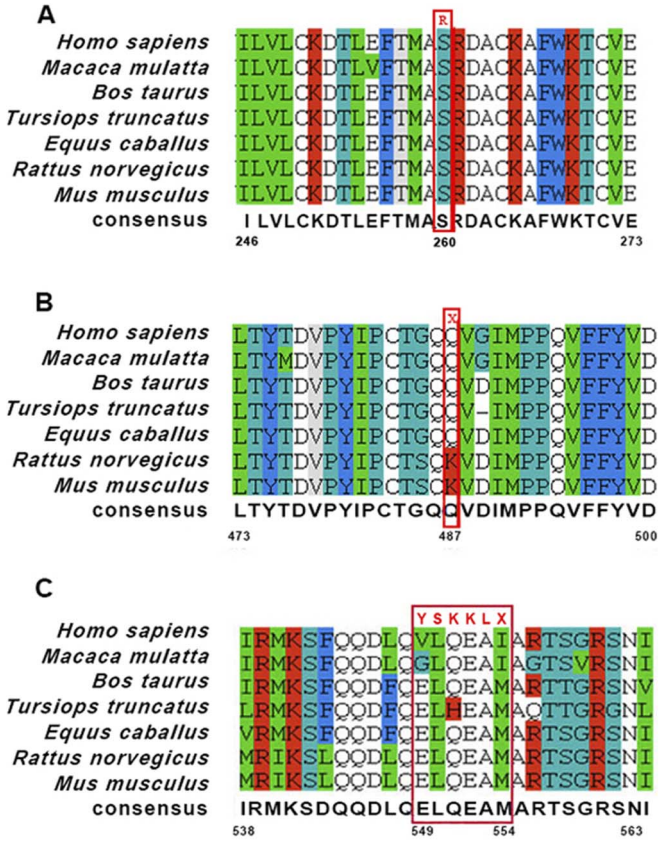

Figure 3 | Multiple-sequence alignment of FRMD7 protein from different species. The red outline in the alignment shows the amino acid affected by the mutation. The deletion mutation (bottom C), which leads to a frameshift mutation, the amino acid residues in red are the residues after frameshift mutation.

Taking this evidence together, we conclude that p.S260R, p.Q487X and p.V549YfsX554 are causative mutations in *FRDM7*, rather than benign polymorphisms in linkage disequilibrium with XLICN.

Discussion

ICN is the most common inherited eye disease manifesting with nystagmus, and patients with this disease usually have problems with visual acuity. Here we recruited four families with XLICN, with the

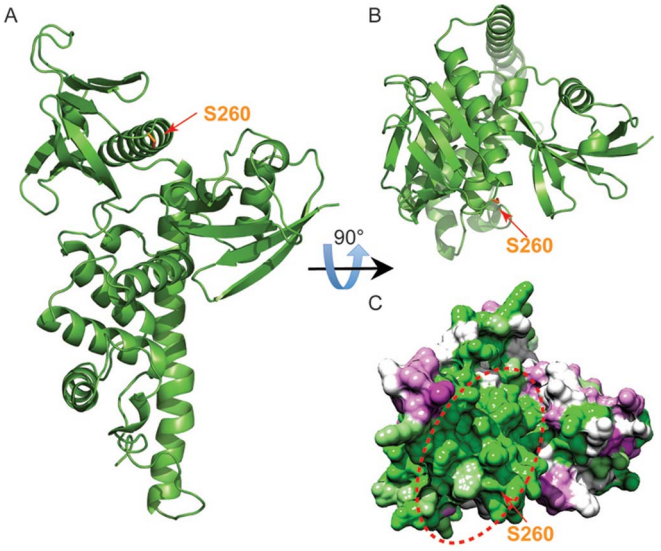

Figure 4 | Structural model of the FRMD7 protein. (A) The 3D model of FRMD7 (1–336) with the key mutation S260 colored in orange. (B) View of FRMD7 after 90 degree rotation. (C) The surface representation of B and the surface is color according to the conservation of the primary sequence. Green regions are strictly conserved, regions in purple are the least conserved areas, and anything in-between is colored white. S260 locates on a large conserved surface.

Table 2 | *FRMD7/GPR143* mutations and best corrected visual acuity(BCVA)

Gene	Mutation	BCVA	Ref.
FRMD7	c.163-1 G > T	20/60–20/25	Hu, Y, 2012
FRMD7	c.673T > G, p.W225G	20/100–20/22	Schorderet DF, 2007
FRMD7	c.780C > A, p.S260R	20/50–20/33	In this study
FRMD7	c.1458G > T, p.Q487X	20/80	In this study
FRMD7	c.1645del G, p.V549YfsX554	20/40	In this study
GPR143	c.658 + 1 G > T	20/200–20/100	Hu J, 2011
GPR143	c.807T > A, p.Y269X	20/2000–20/100	Yan N, 2012
GPR143	c.816del 14 bp	20/200–20/170	Preising M, 2001

goal of identifying the genetic defects present in these families. Since only *FRMD7* and *GPR143* have been reported as causative genes for XLICN, we focused our attention on these two candidates. After comprehensive screening for mutations by direct sequencing, three novel mutations in *FRMD7* were identified but no mutation was identified in *GPR143*. This study thus provides further evidence to show the role of *FRMD7* in the pathogenesis of XLICN, which is consistent with previous evidence that 47% of the XLICN pedigrees in the Chinese population have yielded *FRMD7* mutations[12]. Family D was also recruited but no *FRMD7* or *GPR143* mutations were identified in these individuals. Recently, there was a report that showed *CASK* may be one of the responsible genes for XLICN with mental retardation; we excluded it in the families of this study by capture next generation sequencing. These results further highlight the genetically heterogeneous nature of ICN, since at least four loci have been proposed for familial ICN. It also illustrates the unique role of *FRMD7* in XLICN.

The FRMD7 protein (ENSP00000298542) consists of an N-terminal FERM domain, a FERM adjacent domain and a region without any significant homology to other proteins. The FERM domain is located between amino acids 2–282, and the FERM-C (F3) domain is located at residues 186–279 in FRMD7. Using the Phyre2[13] program, we predicted the secondary structure of the wild-type FRMD7 protein. We modeled the 3D structure of the wild type FRMD7 protein (1–336), and mapped out the S260 in the structure (Figure 4). The p.S260R mutation localizes to the FERM-C domain, and introduces a larger amino acid (arginine) into a restricted area[14]. Since the S260 residue is solvent exposed, we rationalize that the point mutation (p.S260R) may potentially disrupt FRMD7 function through one of the following mechanisms. Firstly, S260 is predicted to be a phosphorylation site, and p.S260R mutation will surely prevent the phosphorylation event, and thus alter the regulation of FRMD7[15]. Secondly, we mapped out the conserved regions of the FRMD7 surface. S260 is located in an extremely conserved surface that usually is involved in protein-protein interactions. It would not be surprising if any point mutation on this surface interferes with the interaction with other proteins, and therefore contribute to the pathogenesis of the disease.

The nonsense mutation and the deletion mutation in *FRMD7* were predicted to cause gross defects at the protein level and loss of the protein function. To date, five mutations[14,16–18] have been reported in exon 12 of *FRMD7*, all of which are predicted to produce truncated proteins. In the present study, two novel mutations in exon 12 were identified. Both are predicted to lead to premature termination of translation. It highlighted that the C-terminal region, which is a highly conserved domain, plays a critical role in the function of FRMD7 protein[11].

The association between best corrected visual acuity (BCVA) and the type of XLICN mutations is of interest, since it may help the clinical genetic diagnosis. Since mutations in *FRMD7* or *GPR143* both can cause XLICN, we reviewed the literature to collect the available data of these two genes mutations and BCVA data, which was summarized in Table 2. We observed that patients with mutations in *GPR143* have worse BCVA[19–21], compared to those with mutations in *FRMD7*[2,22]. Study data is consistent with the reported literature indicating a better BCVA in *FRMD7* compared to *GPR143* patients.

In summary, our findings have further expanded the mutational spectrum of *FRMD7* and further confirmed the genetic heterogeneity of XLICN. By structural modeling, we predicted the consequence of the p.S260R mutation.

Meanwhile, from limited available data, it is suggested that XLICN patients with lower BCVA may have *GPR143* gene mutations. However, the specific molecular consequence of these mutations in *FRMD7* is not clear. Further studies are needed to provide insights into the detailed molecular pathogenesis of the mutations identified in the present study.

Methods

Clinical evaluations and DNA specimens. This study conformed to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Eye Hospital, Wenzhou Medical University. Written informed consent was obtained from all participating individuals or their guardians. Patients from four families (A, B, C and D, Figure 1) originating from the provinces of Helongjiang, Jilin, Inner Mongol and Shandong, respectively, were recruited. Clinical and ophthalmological examinations were performed by ophthalmologists.

Mutation analysis. Primers were designed to cover the sequences of all coding exons and splice junctions of *FRMD7* and *GPR143*. The DNA sequences of *FRMD7* and *GPR143* were obtained from the GenBank database (NM_194277.1 and NM_000273.2). The primer sequences of *FRMD7* have been published[22], and the primers and amplification conditions for *GPR143* are listed in Table 3.

Table 3 | Primers and PCR Conditions for *GPR143* gene

Exon	Primer F (5′-3′)	Primer R (5′-3′)	Annealing temp.	Length (bp)
1	GGCCGGCGGGGTCCTGGCACA	CAGGTCCAAATCCACAGGCC	60°C	360
2	TCCCTCCTTTCCTTCCTTCCTTCT	CCCATGTAAGCGGAGGATGCATTT	62°C	478
3	TCCCTGCATACAAGTGGAGCAGAA	ACAAGCCACTGTGCCTGGCTAATA	62°C	544
4	AAACAACCAACCAACCTCAGCAGC	TGATTCCAGGGTCATTCTCCAAGC	58°C	452
5	CTTTGGGCAGTCAGCAGCAGATTT	TCCTGGCACTGAGCTAACAAACGA	58°C	480
6	TCAGTGACTTGCTTTGCTTCCTGC	CAGCAATGCACATGGTTGGGACAT	58°C	530
7	AAAGTGCTGGGATTACAGGAGGGA	AGGCCAAGACAGAGGATTGCTTGA	68°C	436
8	TTTCAGGCACCCTTGAAGGTACAG	GTGCAACTGGAAGCTAGTGAGT	58°C	612
9	TGCAGATGAGATGAATGCTAGGAC	AGTGTGCTGTAGCCTGTACCATCT	58°C	750

PCR products were sequenced on an ABI PRISM 3730 DNA Sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned and the mutations were detected using Mutation Surveyor software (Soft Genetics, USA, www.softgenetics.com). The effects of the mutations on the protein coding region (synonymous, missense, nonsense, frameshift, etc) were predicted by Exome-assistant[22]. The effects of the mutations on protein function were assessed by four online softwares, including Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), Pmut (http://mmb2.pcb.ub.es:8080/PMut/) and Panther (http://www.pantherdb.org/tools/csnpScoreForm.jsp).

A total of 7080 exons on the X chromosome (Table S2) were selected by a gene capture strategy using the GenCap custom enrichment kit (MyGenostics, Beijing), which has been previously described[23]. The enrichment libraries were sequenced on an Illumina Solexa HiSeq 2000 sequencer for paired read 100 bp. The analysis was described previously[23]. Briefly, using the Solexa QA the cutadapt (http://code.google.com/p/cutadapt/), SOAP aligner, BWA and GATK programs to retrieve and align to identify SNPs and insertions or deletions (InDels). SNPs and InDels were annotated using the exome-assistant program (http://122.228.158.106/exomeassistant). We selected the *CASK* gene (NM_001126054) for the mutation survey.

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Author contributions

F.G. and J.Q. conceived the idea, X.Z., X.L.G., Y.Y., Y.L.Z., Y.M.W. and Y.L. collected the samples and performed the experiments, Z.B.J. and F.G. performed data analyses, X.Z. and J.S. performed structural modeling. F.G. wrote the manuscript. All authors have read and approved the final manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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