Case Report

Novel TRAPPC11 Mutations in a Chinese Pedigree of Limb Girdle Muscular Dystrophy

Xike Wang,1 Yue Wu,1 Yuxia Cui,1 Nan Wang,1 Lasse Folkersen,2 and Yuchuan Wang1

1Department of Pediatrics, Guizhou Provincial People's Hospital, Guiyang, Guizhou 559992, China
2Sankt Hans Hospital, Capital Region Denmark, Roskilde 4000, Denmark

Correspondence should be addressed to Xike Wang; wangxike2008@sina.com

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1. Introduction

More than 50 mutations have been described for LGMD, all having either autosomal-dominant (termed LGMD1) or autosomal-recessive inheritance (termed LGMD2) [1–4]. As such, LGMD constitutes a heterogeneous group of myopathies, but all leading to proximal muscle weakness, with relative sparing of heart and bulbar muscles. The major forms of LGMD result from mutations in genes encoding constituents of the sarcolemmal dystrophin complex, e.g., laminin (LGMD1B), sarcoglycan (LGMD2C-F), and dysferlin (LGMD2B). Other forms have also been described, typically resulting from mutations in genes affecting muscle function involving membrane trafficking [5], muscle remodeling [6], and posttranslational modification of sarcolemmal proteins [7]. The age of onset, severity, and rate of progression vary considerably between LGMD subtypes, ranging from early childhood myopathy to adult onset with long-time preserved ambulation. Here, we reported the clinical and molecular phenotype of an autosomal-recessive form of LGMD caused by two novel TRAPPC11 mutations in a Chinese family of Buyi origin with two affected members.

2. Case Presentation

The first affected individual was from a Chinese family of Buyi origin, a girl born to healthy unrelated parents after an uneventful pregnancy. The affected individual presented at age 5 with a progressive proximal muscle weakness and difficulty in standing up from sitting and walking, particularly stair-climbing. This started at approximately age 2 and gradually worsened. Physical examination was unremarkable except for mild short stature. No other muscle groups, including upper limbs, were affected. Biochemistry analysis revealed more than 100-fold increase of serum creatine kinase.
(CK) levels, markedly elevated serum lactate dehydroge-

nase level (1747 U/L) and α-Hydroxybutyrate dehydrogenase 

(1237 U/L) and mildly elevated serum glutamic-oxaloacetic 

transaminase (142U/L). The electromyogram (EMG) exam-

ination showed a typical muscle-derived damage in both 

affected children. No MRI or muscle biopsies were per-

formed.

2.1. Whole-Exome Sequencing. Genomic DNA samples 

were extracted with Gentra Puregene Kit (Qiagen, Germany) 

from peripheral blood collected from the patient and his parent. 

The quality and quantity of genomic DNA sample were 
determined using a spectrophotometer (NanoDrop, USA). 

Genomic DNA library was prepared with Agilent SureSelect 

Human All Exon Kit v5 reagents, as instructed by the man-

ufacturer's standard protocol. The enriched DNA samples 

were sequenced with Hiseq2000 instrument (Illumina) using 

×2 100 paired-end sequencing. The Illumina Sequencing Con-

trol Software (SCS) v2.8, the Illumina Off-Line Basecaller 

Software (OLB) v1.8, and the Illumina Consensus Assessment 

of Sequence And VARIation (CASAVA) v1.8 were used to 

produce 100 bp sequence reads. The study was approved 

by the Ethics Committee of People’s Hospital of Guizhou 

Province, and written informed consent was obtained from 

their parents of the two affected individuals.

2.2. Alignment, Variant Calling, and Annotation. Burrows-

Wheeler Aligner (BWA) [8] was used to align sequence 

reads to the human reference genome (hg19) with default 

parameters and variants were called using the Genome 

Analysis Toolkit (GATK) software package VarScan [9– 

11]. Coverage was determined using the CalculateHsMet-

rics mode of Picard software. The following analytical 

steps were performed only with reads that matched exonic 

regions including exon-intron-boundaries. SNP and inser-

tion/deletion (indels) analysis was done by different filtering 

steps. The resulting list of variants was annotated with Annovar [12] that summarizes and utilizes information from 

external databases to assess implications and consequences 

of a given sequence alteration, such as amino acid change, 

location within a canonical splice site, and information from 

dbSNP along with the SNP frequency if available. Finally, a 

manual filtering step was carried out to prioritize relevant 

genes in the 30 major LGMD genes for LGMD1B, LGMD2C-

F, and LGMD2B.

2.3. Variants Filtering. The variant detection frequency was 

set with a threshold for variant consideration at a minimum 

of 20% of the reads and an absolute read minimum coverage 

of 10 reads. In each case all variants listed in the most recent 

version of the NCBI (National Center for Biotechnology 

Information) dbSNP database were excluded as well as 

silent mutations. Low frequency frameshift and truncating 

mutations in any LGMD gene were considered pathogenic. 

Unreported nonsynonymous amino acid variants were 

analyzed by MutationTaster (http://www.mutationtaster.org), 

Polyphen-2 (http://genetics.bwh.harvard.edu/pph2), and 

SIFT (http://sift.jcvi.org) to assess any potentially damaging 

effect. Variants passing these filtering steps were considered 
to be most likely disease-causing and forwarded to validation 

process by Sanger sequencing. Additionally, genes with at 

least two heterozygous changes in the DNA sequence were 

considered to be most likely disease-causing, even though 

homozygous variants were not completely withdrawn.

2.4. Sanger Sequencing. We validated the candidate vari-

ations by Sanger sequencing in the two affected individuals 

and their parents. PCR primers were designed with Primer3 
tool (http://frodo.wi.mit.edu/primer3/) to contain the mutation 
sites and their flanking regions (PCR primers and PCR reaction conditions are available upon request). PCR 

amplifications were inspected for single band of expected 
sizes on agarose gels before purification with Agencourt 

AMPure on Biomek NX (Beckman Coulter, USA). Sequenc-

ing was achieved using the automated ABI Prism 3730xlDNA 

Sequencer in combination with the Big Dye Terminator Cycle 

Sequencing Ready Reaction Kit 3.1 (Applied Biosystems, 

USA), and purification of sequencing reaction was performed 

with Agencourt CleanSEQ on Biomek NX (Beckman Coulter, 

USA). Sequences were assembled and analyzed with Muta-

tion Surveyor software (SoftGenetics, USA).

3. Results

Exon-wide sequencing revealed two heterozygous mutations 
in exon 11 and exon 27, respectively, of TRAPPC11 in both 

affected children. Both of them were single base substitu-


Sanger sequencing confirmed the two heterozygous muta-

tions in the patients and each was inherited from one of 

the parents (c.1192C>T (p.Arg398*) from the mother and 

c.3014C>T (p.Pro1005Leu) from the father (Figure 1). The 

two mutations were found to be conserved across multiple 

species and in known functional domains of the TRAPPC11 
gene (Figure 2).

So far, there were only four mutations in TRAPPC11 
reported in patients. The two mutations detected in 

our patients have not been described in patients. The 
c.1192C>T (p.Arg398*) was observed as rs140403642 in 

the NHLBI GO Exome Sequencing Project database (ESP, 
http://evs.gs.washington.edu/EVS/) with a MAF of 0.000077, 
c.3014C>T (p.Pro1005Leu). The variants were found as in 

the ExAC database in 1 and 5 samples, corresponding to a 
frequency of 8.2e-06 and 4.1e-05, respectively. In ExAC they 

were not detected in non-Caucasian ethnicity samples and 

thus represent novel mutations in Asian ethnicity. Likewise, 

they were not found in the 1000 genomes database and they 

were undescribed in the ClinVar database.

4. Discussion

In this report, we identified by whole-exome sequenc-

ing a compound heterozygous mutations in TRAPPC11 
(AK022778.1), i.e., c.1192C>T (p.Arg398*) and c.3014C>T 
(p.Pro1005Leu), in two affected members of a Chinese 

family with LGMD. Previous findings include the whole-

exome sequencing combined with linkage analysis of a
Syrian family with limb girdle muscular dystrophy type 2S (LGMD2S; OMIM# 615356). Further, Bogershausen et al. identified a homozygous mutation in the TRAPPC11 gene (G980R) [13]. The authors also detected a different homozygous mutation in the TRAPPC11 gene (Ala372 Ser429del) in affected members of 2 Hutterite families with a slightly different phenotype. The G980R mutation occurred in the gryzun domain, whereas the deletion occurred in the foie gras domain. Similarly, c.1192C>T (p.Arg398*) mutation and c.3014C>T (p.Pro1005Leu) mutation reported in this study also occurred in the foie gras domain and the gryzun domain, respectively (Figure 2). Recent additional work further expands the breadth of findings, as summarized in Table 1 [14–16].

The affected individuals with TRAPPC11 mutations were described with two groups of clinical manifestations: one with more prominent muscular and skeletal symptoms and the other with microcephaly, hyperkinetic movements,
**Table 1:** Comparison of the present patient and previously reported patients with TRAPPC11 mutations. NR: not reported.

<table>
<thead>
<tr>
<th></th>
<th>c.2938G &gt; A homo</th>
<th>c.1287 + 5G &gt; A homo</th>
<th>c.2938G &gt; A, c.661-1G &gt; T</th>
<th>c.1893+3A&gt;G, g.4184,607,904A&gt;G</th>
<th>c.142C&gt;T, g.27324T&gt;A</th>
<th>c.2330A&gt;C, c.513-516 (delTTTG)</th>
<th>c.1192C&gt;T, c.3014C&gt;T</th>
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<tr>
<td>Number of patients</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Age of onset of muscle symptoms</td>
<td>Early school age</td>
<td>Early childhood onset</td>
<td>Around 1-year-old or even earlier</td>
<td>NR</td>
<td>NR</td>
<td>Early childhood onset</td>
<td>Approx. 2-year-old</td>
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<td>Muscle pathology</td>
<td>Proximal weakness, myalgia, cramps Myopathic</td>
<td>Mild weakness and hypotonia, myopathic</td>
<td>Weakness, Dystrophy, Atrophy</td>
<td>Hypotonia</td>
<td>Mild weakness and hypotonia, myopathic</td>
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<td>CK (IU/L)</td>
<td>600–2800</td>
<td>300–1000</td>
<td>&lt;3rd percentile</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Head circumference</td>
<td>Within normal limits</td>
<td>&lt;3rd percentile</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
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<td>Intellectual disability</td>
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<td>(+)</td>
<td>Borderline</td>
<td>(+)</td>
<td>(+)</td>
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<td>(+)</td>
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<tr>
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<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
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<td>(-)</td>
<td>Generalized seizure Abnormal EEG</td>
<td>(-)</td>
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<td>(-)</td>
<td>Generalized seizure, Abnormal EEG</td>
<td>(-)</td>
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<td>Neuroimaging</td>
<td>Enlarged right ventricle</td>
<td>Mild cerebral atrophy</td>
<td>Reduced white matter volume</td>
<td>Cerebral atrophy NR</td>
<td>Brachycephaly NA</td>
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<td>Cardiac involvement</td>
<td>Hip dysplasia, scoliosis</td>
<td>Limb asymmetry</td>
<td>Lordosis (-)</td>
<td>Scoliosis, skeletal anomalies (-)</td>
<td>(-)</td>
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<td>Skeletal involvement</td>
<td>Exotropia, anisometropia, and amblyopia</td>
<td>Infantile—onset cataract</td>
<td>(-)</td>
<td>NR</td>
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<td>Steatosis (-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Hepatic involvement</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<td>(13)</td>
<td>(14)</td>
<td>(15)</td>
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ataxia, and intellectual disability, which was discussed as a reflection of the difference of the two genotypes, Gly980Arg and Ala372Ser429del [13]. Most recently, Liang et al. reported a Chinese girl harboring a compound heterozygous c.2938G > A/c.661-1G > T mutations in TRAPPC11 presenting congenital muscular dystrophy, fatty liver, and infantile-onset cataract, demonstrating the broad spectrum of disease phenotypes arising from TRAPPC11 mutation in human [17]. The affected individuals from the Chinese family of LGMD2S reported herein were mainly characterized with progressive proximal muscle weakness resulting in impaired ambulation, difficulty to climb stairs, and increased serum creatine kinase. We did not observe noticeable features of nervous and hepatic involvement in the two patients with LGMD2S. The results clearly revealed that there is significant variability in phenotypes of TRAPPC11 mutations.

TRAPPC11 is a component of the TRAPP multisubunit tethering complex involved in intracellular vesicle trafficking [18]. Patient cells from both groups showed increased fragmentation of the Golgi apparatus and decreased amounts of the mutant proteins. Studies in yeast suggested that the mutant missense protein lost the ability to interact properly with other TRAPP proteins. Patient cells also showed altered protein transport along the secretory pathway, with a delayed exit from the Golgi and a defect in the formation and/or protein transport along the secretory pathway, with a delayed exit from the Golgi and a defect in the formation and/or movement of late endosomes/lysosomes [13]. Liang et al. did not observe full-length TRAPPC11 protein in the patient harboring c.2938G > A/c.661-1G > T compound heterozygous mutations [17]. The findings suggested that altered membrane trafficking is the underlying molecular mechanism of this disease spectrum. Interestingly TRAPPC11 has also been associated with glycosylation [19] and later studies on zebra fish have confirmed that TRAPPC11 is involved in protein glycosylation [20], providing for a more detailed hypothesis on disease mechanism in this case report.

In conclusion, this study widens the phenotype of TRAPPC11 mutation related disorder and provides a conclusive case report for LGMD.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References


