Exome sequencing identifies a novel mutation of the GDI1 gene in a Chinese non-syndromic X-linked intellectual disability family

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Abstract

X-linked intellectual disability (XLID) has been associated with various genes. Diagnosis of XLID, especially for non-syndromic ones (NS-XLID), is often hampered by the heterogeneity of this disease. Here we report the case of a Chinese family in which three males suffer from intellectual disability (ID). The three patients shared the same phenotype: no typical clinical manifestation other than IQ score ≤ 70. For a genetic diagnosis for this family we carried out whole exome sequencing on the proband, and validated 16 variants of interest in the genomic DNA of all the family members. A missense mutation (c.710G > T), which mapped to exon 6 of the Rab GDP-Dissociation Inhibitor 1 (GDI1) gene, was found segregating with the ID phenotype, and this mutation changes the 237th position in the guanosine diphosphate dissociation inhibitor (GDI) protein from glycine to valine (p.Gly237Val). Through molecular dynamics simulations we found that this substitution results in a conformational change of GDI, possibly affecting the Rab-binding capacity of this protein. In conclusion, our study identified a novel GDI1 mutation that is possibly NS-XLID causative, and showed that whole exome sequencing provides advantages for detecting novel ID-associated variants and can greatly facilitate the genetic diagnosis of the disease.

Keywords: Intellectual disability, GDI1 gene, guanosine diphosphate dissociation inhibitor, whole exome sequencing.

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Introduction

Intellectual disability (ID) is a neurodevelopmental disorder that appears before the age of 18. Its main clinical manifestations are intellectual deficits and social adjustment problems (Raymond, 2006). The average prevalence of ID in the last two decades was 13.0 per 1,000 in eight-year-old children in Atlanta, USA (Van Naarden Braun et al., 2015), and 7.5 per 1,000 in the general population in China during the same period (Wu et al., 2010). Impaired cognitive ability and social adaptation difficulties make it difficult for ID patients to live independently. Thus, the patients usually need lifelong care at home or in welfare centers, which pose enormous socioeconomic burdens for their family and the society (van Schrojenstein Lantman-de Valk and Walsh, 2008).

ID may arise from environmental factors, genetic predisposition, or a combination of both. In addition, the clinical manifestations of ID are highly heterogeneous, which makes it difficult to confirm the etiology of most ID patients by traditional clinical diagnostic processes (Stevenson et al., 2003). As to the cases in which genetic factors play a role, a great variety of chromosomal abnormalities and gene mutations are involved (Inlow and Restifo, 2004). Hitherto, there are approximately 820 genes considered responsible for ID (Kochinke et al., 2016). Examining all of those genes in each ID case by Sanger sequencing is impractical. Therefore, there are increasing needs for new technical improvements to make precise molecular diagnosis for inherited ID patients. Nowadays, the next-generation sequencing (NGS) technology provides advantages for the genetic diagnosis of ID. The application of exome sequencing, a variant of NGS that focuses on the coding regions of the genome, improves the efficiency of molecular diagnosis and helps to uncover novel mutations present in either sporadic or familial cases with non-specific phenotypes (Topper et al., 2011; de Ligt et al., 2012; Rauch et al., 2012).

Here, we present the genetic diagnosis of a Chinese family of Han origin with three males suffering from non-syndromic X-linked intellectual disability (NS-XLID) carried out by whole exome sequencing (WES). To our knowledge, this family might be the fifth XLID case caused by...
mutations located in the Rab GDP-Dissociation Inhibitor 1 (GDI1) gene (OMIM*300104) ever reported (Strobl-Wildemann et al., 2011).

Materials and Methods

Ethical statement

This study was approved by the Ethics Committee of Shenzhen Research Institute of Population and Family Planning (SZIPP) in accordance with the Declaration of Helsinki (review list No. 20150411001). Written informed consents for publication of clinical information and genetic investigation were obtained from each participant or their legal guardians.

Clinical information of patients and family ascertainment

This family, coded SZMRX, is of Chinese Han origin, with three generations including six members and one fetus (Figure 1A). There are three affected males belonging to two generations. The proband (III:1) is a 5-year old male, who was initially noted to have language developmental delay at 3 years old. His spoken language was limited to single words, like “father” and “mother”, and his emotions were expressed with purposeful body movements in most times. He was born after normal pregnancy and spontaneous delivery course, and his growth parameters were within the normal ranges of the Chinese reference for children’s growth. He started to walk at the age of 18 months and his muscle tonus was within normal. Clinical, physical and mental examinations showed only a moderate intellectual disability, with verbal intelligence quotient (VIQ) = 44, performance IQ (PIQ) = 55, and full scale IQ = 45, evaluated by the Wechsler Preschool and Primary Scale of Intelligence III (WPPSI-III). The two maternal uncles of the proband (II:1 and II:3) presented the same phenotype: retardations were apparent in the first three years of life and the development of intelligence was non-progressive; there was no typical clinical manifestation other than the limitations in intellectual function and adaptive behaviors. The cranial magnetic resonance imaging (MRI) examination was performed in the 3 patients of this family and no abnormal anatomical feature was detected. Chromosomal aberrations and fragile X-syndrome were ruled out by G-banding karyotype examination and FMR1 mutation analysis (data not shown).

All obligate and possible carriers were of normal intelligence, and the pregnancy and delivery courses were uneventful in all female members. The mother of the proband got pregnant two years after his birth, but she terminated her pregnancy at the 12th week for personal reasons. She was pregnant recently and the amniocentesis was executed at the 18th week of this pregnancy. The pathological phenotype is considered inherited in X-linked recessive mode for several reasons. First, multiple affected members exist in continuous two generations reveal that the ID phenotype of the proband is not from a de novo mutation. Second, the normal phenotype of the proband’s parents demonstrates that the ID phenotype is not inherited in an autosomal-dominant or X-linked dominant mode. Third, in consideration of the relatively rare allele frequency of ID-causing mutations, autosomal-recessive causes would be rather unlikely, as several individuals from two generations of this family with no consanguineous marriage were affected.

Figure 1 - Pedigree and mutation. (A) Pedigree of the SZMRX Family. (B) Schematic of GDI1 gene; arrow shows the c.710G > T mutation located at exon 6 of the GDI1 gene (chr X: 153,668,844). C. The c.710G > T mutation segregating with the phenotype of non-syndromic X-linked intellectual disability (NS-XLID) in all male patients (II:1, II:3 and III:1) was validated by Sanger sequencing; arrow shows the location of c.710 (chr X: 153,668,844).
DNA isolation

Genomic deoxyribonucleic acid (gDNA) was isolated from peripheral blood of the proband and all family members, and from the amniotic fluid of the fetus using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany).

Whole exome sequencing

The proband underwent WES investigation, which was carried out on an Ion Torrent PGM platform (Life Technologies, Carlsbad, CA, USA). In brief, gDNA of the proband was fragmented by Ion Shear Plus Reagents to generate the fragment library. Exome capture was conducted by hybridizing the fragment library with biotin-labeled blocker at 47 °C for 72 h, followed by extraction using streptavidin-coated magnetic beads. Then the exome-enriched library was amplified by Ion TargetSeq Amplification Primer and purified using Agencourt AMPure XP Reagent (reagents mentioned above were all from Ion Plus Fragment Library Kit, Life Technologies). Thereafter, the template was produced by emulsion PCR (Ion PGM Template OT2 200 Kit) using the Ion OneTouch System (Life Technologies), and the sequencing program was executed on Ion 318 Chip V2 using the Ion PGM Sequencer (Life Technologies).

Data analysis and validation

The whole exome sequencing data obtained from the Ion Torrent PGM platform were qualified as 98.76% of the exonic bases covered by at least 1 read, and 87.45% of those covered by 10 reads or more. The sequenced reads were aligned using the human reference genome (GRCh37/hg19) as the reference sequence, then the aligned reads were applied to call variants by TVC4.2 and annotated by Ion Reporter 4.4 (Life Technologies). The alternative allele frequency (AAF) of all the variants was further adjusted by the self-written script to browse the 1000 Genomes Project database, and common variants were excluded by filtering out those AAF > 0.01, in other words, only rare mutations were left for further investigation. Next, a script was executed to reserve only dangerous mutations, which were defined as variants that probably disrupt protein functions, such as frame shift indels, splice site variations, stop gain or loss, and deleterious missense (SIFT score < 0.05 or PolyPhen score > 0.5) (De Rubeis et al., 2014). Finally, all the in silico predicted dangerous mutations were validated by Sanger sequencing in the proband, all family members and the fetus as follows. The locations of interest in genomic DNA were amplified by PCR using primers designed by Primer Premier 5.0 (PREMIER Biosoft), shown in Supplementary Table S1. Sanger sequencing was carried out using the ABI Prism Big Dye Terminator Cycle Sequencing v3.1 Kit (Applied Biosystems, Foster City, CA, USA) on an ABI-3130xl genetic analyzer (Applied Biosystems). Co-segregation analysis was performed and mutations of X-linked recessive mode inheritance were considered because of the multiple affected members and the normal phenotype of the proband’s parents.

In silico analysis of the impact of amino acid conversion on the structure and molecular dynamics of GDI protein

The amino acid homology sequence alignment was performed using ClustalX to verify the evolutionary conservation of amino acid sequences we concerned between several vertebrates and human (Larkin et al., 2007). The structure modeling work of both wild type and mutated protein was done by Modeller 9.11 on the basis of bovine GDP-Dissociation Inhibitor α-isofrom (PDB ID: 1DST), and assessed by SAVES. The structural effects of interested amino acid conversion were predicted by HOPE (Have (y)Our Protein Explained) website (Venselaar et al., 2010). Molecular dynamics simulations of both wild type and mutated protein were conducted using a version of the PMEMD module from AMBER 12 (Case et al., 2005).

Results

Sixteen candidate variants (15 homozygous and 1 heterozygous, located in 12 genes) were identified from the proband by analysis of the WES data (Table 1). Subsequent Sanger validation followed by co-segregation analysis detected a c.710G > T (Chr.X: 153, 668, 844G > T) missense mutation, which mapped to exon 6 of the GDI1 gene in all male patients (II: 1, II: 3 and III: 1), segregating with the phenotype of NS-XLID (Figure 1B, C). Meanwhile, the proband’s mother and grandmother, who have a normal intellectual phenotype and social adjustment ability, were proved to be obligate carriers of this mutation (Figure 1C). Additionally, we also checked this variant by comparing to ExAC and 5000 Exomes (for URLs see the Internet Resources), and insured that they were not present in either of these two large scale exome consortia.

This missense mutation changes the 237th position amino acid in the alpha-isofrom GDP-Dissociation Inhibitor protein from glycine to valine (p.Gly237Val). Amino acid sequence alignment by ClustalX showed that the region, which p.Gly237Val mutant residue was situated in, was conserved across various vertebrates. Namely, neither this mutant residue (Val) nor any other residue with similar properties was observed at this position in other homologous sequences of vertebrates (Figure 2A). Several pathogenicity prediction score programs gave deleterious predictions for this p.Gly237Val mutation (Table S2). The computer built model of the GDI protein revealed that the location of residue 237 belongs to a hydrophobic domain composed by four helices, and substitution of glycine with valine introduces a larger side-chain into this four-helix hydrophobic pocket (Figure 2B). Molecular dynamics simulations lasting 50 nanoseconds (ns) also revealed that the residue 237 located in the helix of wild type αGDI exhibited a distinct conformational change after the minimi-
zation, heating and equilibration procedures (Figure 3A), while the p.Gly237Val mutant showed a relatively less obvious conformational change (Figure 3B).

**Table 1 - Candidate mutations of the SZMRX family identified by whole exome sequencing.**

| Gene      | Genomic Positions (hg19) | Variant Type | Genotype | Nucleotide changes | Protein changes | Status        |
|-----------|-------------------------|--------------|----------|--------------------|----------------|---------------|
| GALE      | chr1:24,123,434         | SNV          | homozygous | Splice site 5. T>G  |                | Not co-segregating |
| IDUA|SLC26A1 | chr4:983,625 | SNV | homozygous | c.1102 G>A | p.Gly368Ser | Not co-segregating |
| NDST1     | chr5:149,907,466        | SNV          | homozygous | c.614 C>T         | p.Pro205Leu    | Not co-segregating |
| SLC17A5   | chr6:74,331,619         | SNV          | homozygous | c.886 G>A         | p.Val296Leu    | Not co-segregating |
| AH1       | chr6:135,611,614        | SNV          | homozygous | c.3535 G>T         | p.Asp1179Tyr   | Not co-segregating |
| TG        | chr8:133,931,735        | SNV          | homozygous | c.4493 C>T         | p.Thr1498Met   | Not co-segregating |
| DOK8      | chr9:312,134            | SNV          | homozygous | c.709G>A           | p.Glu237Lys    | Not co-segregating |
| FANCC     | chr9:97,887,391         | SNV          | homozygous | c.973G>A           | p.Ala325Thr    | Not co-segregating |
| ABC8      | chr11:17,483,176       | INDEL        | homozygous | c.775_775delG       | p.Ala259frame shift | Not co-segregating |
| SLC35C1   | chr11:45,832,441        | SNV          | homozygous | c.611C>T           | p.Thr204Met    | Not co-segregating |
| TMEM216   | chr11:61,165,741        | SNV          | heterozygous | c.440G>C           | p.Arg147Thr    | Not co-segregating |
| KMT2D     | chr12:49,434,409        | SNV          | homozygous | c.7144C>T          | p.Pro2382Ser   | Not co-segregating |
| TSC2      | chr16:2,133,765         | SNV          | homozygous | c.3953A>G          | p.Glu1318Gly   | Not co-segregating |
| CTSA/PLTP | chr20:44,526,704        | SNV          | homozygous | c.1369G>A          | p.Gly457Ser    | Not co-segregating |
| COL1A1(MIR6815) | chr21:46,898,266 | SNV | homozygous | c.1787C>T         | p.Pro596Leu    | Not co-segregating |
| GDI1      | chrX:153,668,844        | SNV          | homozygous | c.710G>T           | p.Gly237Val    | Co-segregating (X-linked recessive) |

#: Sixteen candidate mutations (located in 12 genes) were identified from the proband by analysis of the whole exome sequencing data, and finally a c.710 G>T (chrX: 153,668,844G>T) missense mutation, which mapped to exon 6 of the GDI1 gene (OMIM* 300104), was confirmed to be a pathogenic variant by Sanger sequencing and co-segregation analysis in all male patients (II:1, II:3 and III:1).

**Discussion**

Here we report a family (SZMRX) with three patients suffering from intellectual disability. Ultimately, the inheritance pattern of ID in this family was identified to be an X-linked recessive type by the WES and subsequent Sanger validation followed by co-segregation analysis, as the two unaffected females (mother-daughter relationship) share one rare heterozygous mutation related to XLID, and three consanguineous male patients are homozygous of the same loci.

The unique phenotype presented in the three patients is a moderate ID without any other recognizable clinical signs, and therefore the potential cause of this phenotype cannot be determined by G-banding karyotype examination and FMR1 mutation analysis. Whole exome sequencing was performed for the proband, and the probable pathogenic variants were validated by Sanger sequencing for all the family members and the fetus. Analysis of the WES data and subsequent Sanger validation followed by co-segregation analysis showed a missense mutation that segregated with the ID phenotype. This variant is a G to T transversion at position 710 of the coding sequence (c.710G>T), which is mapped to exon 6 of the GDI1 gene, and it results in a p.Gly237Val substitution in the encoded protein. The GDI1 gene contains 11 exons, spans 6.29 kb, and is located at chr. Xq28. This gene encodes the protein GDI, which belongs to the TCD/MRS6 family of GDP dis-
The 237 mutation. (A) The 237 residue located helix of the wild type αGDI exhibited a distinct conformational change after a 50 ns molecular dynamics simulation. (B) The conformation of this helix of 237 mutant αGDI showed relative stability vs. the wild type. The red color represents the initial conformation of 237 residue located helix of both wild type and 237 mutant αGDI, and green indicates the ones simulated by molecular dynamics.

Figure 3 - The 237 mutation. (A) The 237 residue located helix of the wild type αGDI showed a relative stability vs. the wild type. The red color represents the initial conformation of 237 residue located helix of both wild type and 237 mutant αGDI, and green indicates the ones simulated by molecular dynamics.
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Internet Resources

5000 Exomes, http://evs.gs.washington.edu/EVS/ (December 15, 2015).

ExAC, http://exac.broadinstitute.org/ (December 15, 2015).

HOPE, http://www.cmbi.ru.nl/hope/ (January 11, 2016).

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Supplementary material

The following online material is available for this article:

Table S1 - Characteristics of primers for amplification of candidate pathogenic regions on the genomic DNA

Table S2 - Possible consequence of mutations predicted by various algorithms

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