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Brief Communication

Identification of a novel nonsense homozygous mutation of *LINS1* gene in two sisters with intellectual disability, schizophrenia, and anxiety

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ABSTRACT

Background: *LINS1* encodes the lines homolog 1 protein that contains the *Drosophila lines* homologous domain. *LINS1* mutations cause a rare recessive form of intellectual disability. So far, eight *LINS1* mutations were reported in the literature.
Methods: We conducted a whole-genome sequencing analysis for a family with two sisters diagnosed with moderate intellectual disability, schizophrenia, and anxiety.
Results: We identified a novel homozygous nonsense mutation in the *LINS1* in these two sisters. The mutation was a C-to-T substitution at the cDNA nucleotide position 274 that changed the amino acid glutamine at the codon 92 to stop codon (Gln92X). The mutation was transmitted from their unrelated parents, who were heterozygous carriers.
Conclusions: We identified the first case of *LINS1*-associated neurodevelopmental disorder in Taiwan. Our findings suggest that besides intellectual disability, psychiatric diagnoses such as schizophrenia and anxiety disorder may also be part of clinical phenotypes of *LINS1* mutations.

Intellectual disability (ID) is a childhood-onset developmental disorder defined by the reduced intelligent function that leads to impaired learning ability and social adaptation. The prevalence of ID is approximately 1% in the general population worldwide [1]. ID is a complex disorder with the genetic factor as the leading cause [2]. Genetic mutations of ID are highly heterogeneous, ranging from chromosomal abnormalities, copy number variations to single-gene mutations [3,4]. ID is further divided into syndromic and non-syndromic, depending on whether there is a co-existence of distinguishable physical abnormalities [2]. Several hundreds of genes associated with ID have been identified. However, many more remain to be identified [2,5]. It is challenging to establish the correct genetic diagnosis for the affected patients to offer genetic counseling and guide the clinical treatment.

The *LINS1* is located at 15q26.3 and encodes the lines homolog 1 protein that contains the *Drosophila lines* homologous domain [6]. Mutations of *LINS1* are very rare, and they are linked to a recessive type of intellectual disability. In a study of 136 consanguineous families with autosomal-recessive ID, a homozygous deletion of four nucleotides in exon 5 of *LINS1* was detected in one family [7]. In another study, a homozygous splicing mutation of *LINS1* was identified in two children with ID in a consanguineous family [8]. Furthermore, a

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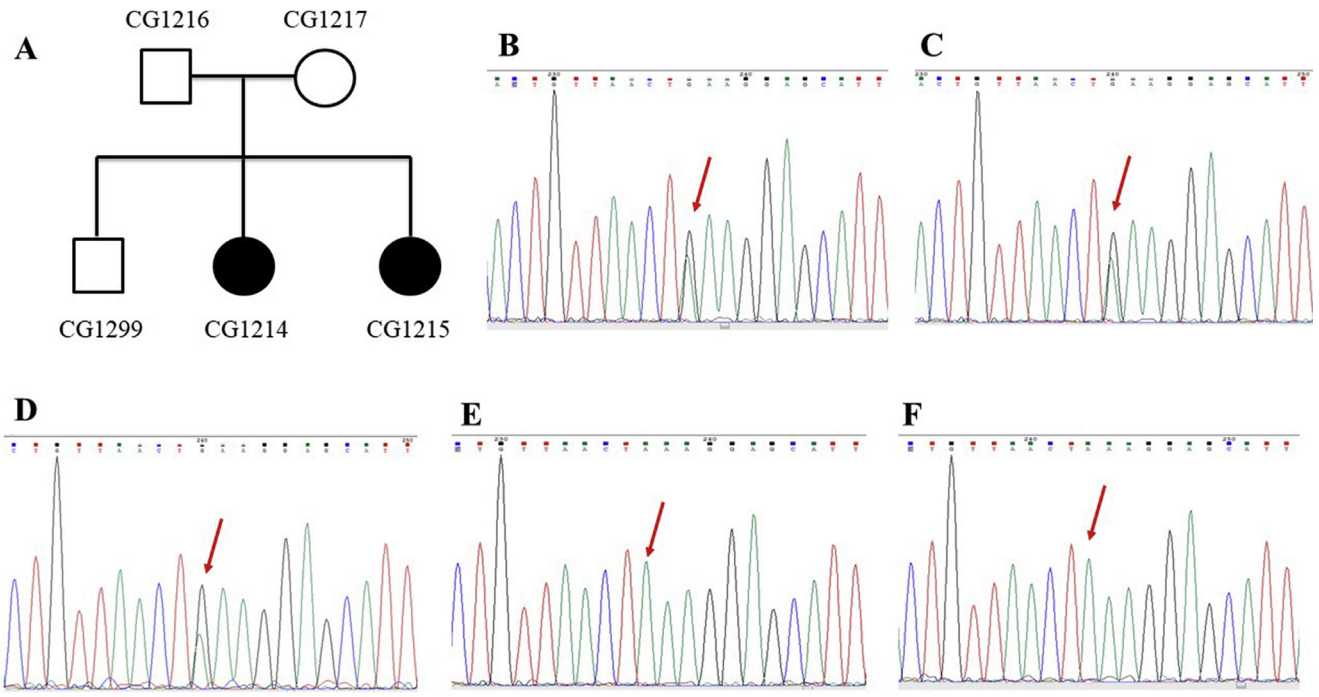

Fig. 1 Pedigree and chromatograms of the Gln92X mutation of the *LINS1* identified in this study. (A) Pedigree of family. (B) Chromatogram of the father (CG1216) who is a heterozygous carrier of the G-to-A mutation. (C) Chromatogram of the mother (CG1217) who is also a heterozygous carrier of the G-to-A mutation. (D) Chromatogram of the son (CG1299) who is a heterozygous carrier of the G-to-A mutation. (E) Chromatogram of the elder sister (CG1214) who is a homozygote of the G-to-A mutation. (F) Chromatogram of the younger sister (CG1215) who is also a homozygote of the G-to-A mutation. The red arrow indicates the position of nucleotide change.

homozygous missense mutation E313K of *LINS1* was reported in two siblings presenting non-syndromic ID and mutism [9]. A homozygous nonsense mutation (Q368X) of *LINS1* was recently found in two siblings with non-syndromic ID in a consanguineous family [10]. In China, a homozygous single nucleotide deletion of *LINS1* that led to frameshift mutation (D241fs) of *LINS1* was identified in a child with severe ID [11]. A homozygous eight bp deletion of *LINS1* was reported in three patients with ID and complex neurological deficits from a consanguineous family [12]. Together, these data indicate that loss-of-function mutations of *LINS1* cause a recessive form of non-syndromic intellectual disability.

We recruited a family with two affected sisters who were diagnosed with moderate ID since childhood. These two sisters started to manifest psychiatric symptoms meeting the diagnosis of schizophrenia and anxiety in their thirties. We conducted a whole-genome sequencing analysis for this family and detected a novel nonsense homozygous mutation of *LINS1* in these two sisters.

Materials and methods

Subjects

We recruited a Taiwanese nuclear family of five members from the outpatient clinic of the Department of Psychiatry, Chang-Gung Memorial Hospital-Linkou, Taoyuan, Taiwan. The study was approved by the Institutional Review Board of Chang-Gung Memorial Hospital with the approved number 201801385A3. We obtained informed consent from each subject after a full explanation of the study. Genomic DNA was prepared from each subject.

Whole-genome sequencing

Paired-end whole-genome sequencing was performed using the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) according to the standard protocol provided by the company. After a quality check, the raw data were aligned to the human reference genome build hg19. SAMtools and Genome Analysis Tool Kit were used to refine the local alignment and generate variant calling files (VCF) for each subject. Variants were further annotated, filtered, and analyzed under different inheritance models. The bioinformatics and family analyses were implemented using SeqsLab software (Atgenomix, Taipei, Taiwan).

Sanger sequencing

We designed a pair of primers (LINS1-F:5′-GCA CTG TGC AGC CAT GTG AGA-3′ and LINS1-R:5′-GGG CAA ACA CCT GTG GTA TCC-3′) to obtain an amplicon of 399 bp that covered the mutation by polymerase chain reaction (PCR). An aliquot of the amplicon was sequenced using the BigDye Terminator kit v3.1 (Applied Biosystems, Foster, CA, USA).

Bioinformatics analysis

The frequency of the mutation identified in this study was checked in the dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and the Taiwan Biobank (https://taiwanview.twbiobank.org.tw/index). The prediction of the functional impact of the nonsense mutation identified in this study was assessed using the web-based MutationTaster 2 (http://mutationtaster.org/).

Results

Clinical findings

The pedigree of this family is shown in Fig. 1A. The unrelated parents were in their sixties. They had an unaffected son in his forties, while two daughters in their forties were diagnosed with moderate ID since childhood. Both sisters were born at full term with uneventful pregnancies. However, they had a delay in developmental milestones, especially speech development. They were diagnosed with moderate ID with unknown etiology since childhood. They did not have other physical abnormalities nor seizure history. They received special education from elementary to middle school, but they had a poor social function. Both sisters started to manifest psychotic symptoms such as auditory hallucinations, self-talking, delusions of persecution, bizarre behaviors, and wandering in their thirties. Also, they had severe anxiety in addition to their psychotic features. Hence, their diagnosis was moderate ID comorbid with schizophrenia and anxiety disorder. They received treatment of antipsychotics and anxiolytics with good responses. Currently, they remained stable under long-term medication.

Whole-genome sequencing and Sanger sequencing

Under the recessive inheritance, we identified a G-to-A homozygous mutation at the genomic DNA position 101,120,774 (hg19) of *LINS1* in both sisters. This mutation led to a C-to-T substitution at the cDNA position 274, resulting in an amino acid change from glutamine to stop codon at the protein sequence 92, designated Gln92X. The mutation was inherited from their parents, who were heterozygous of this mutation. The eldest son was also a heterozygous carrier of this mutation. The authenticity of this mutation was confirmed by Sanger sequencing. The chromatograms of the mutation in this family are shown in Fig. 1B–F.

Bioinformatics analysis

This G-to-A mutation was present in the dbSNP and assigned rs755894515. The allele frequency of this mutation in the current release (20,201,027,095,038) of the Allele Frequency Aggregator (ALFA) is 0. It is also very rare in the Genome Aggregation Database (GenomAD_exome, 0.000080) and the Exome Aggregation Consortium (ExAC, 0.000074), while the frequency of this allele in Taiwan Biobank is 0.002. The web-based software MutationTaster 2 predicted that the nonsense mutation was disease-causing.

Discussion

This study identified a novel homozygous mutation of *LINS1* (Gln92X) in two sisters diagnosed with moderate ID since childhood. They did not have seizure history or other distinguishable physical abnormalities or facial dysmorphisms. Hence, they belong to non-syndromic ID. To our knowledge, seven studies reported the developmental conditions associated with *LINS1* mutations in the literature [7–12]. All the reported patients shared a global developmental problem and non-syndromic ID, given some variations in clinical phenotype. So far, a total of nine mutations of *LINS1* were identified from these studies, including ours. All these mutations are rare mutations and private for each affected family, suggesting the high allelic heterogeneity of

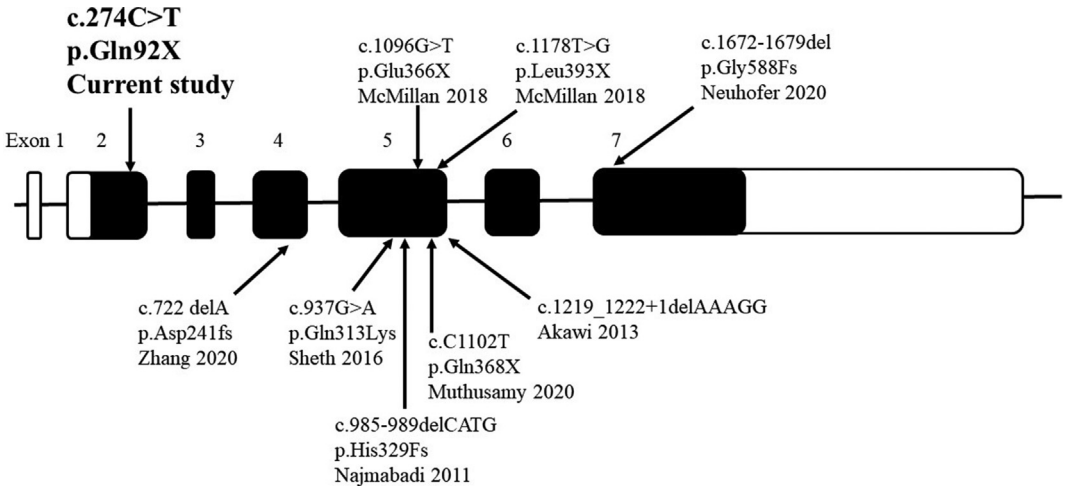

Fig. 2 Schematic locations of mutations of the *LINS1* gene associated with a recessive form of intellectual disability reported in the literature. The Gln92X mutation (in bold) located at exon 2 is the novel one identified in the present study.

LINS1 mutation-associated ID. We summarize the positions of these mutations in Fig. 2.

Notably, our patients developed schizophrenia and anxiety disorder in their thirties. They did not have behavioral problems that need psychiatric intervention before the onset of their psychiatric conditions. Approximately one-third of the people diagnosed with ID have co-occurrence of psychiatric disorders, with the over-representation of the diagnosis of schizophrenia. In *LINS1*-associated ID, behavioral problems such as hyperactivity, aggression, and autistic features were mentioned in some but not all patients [8,9]. To our knowledge, we are the first to report the co-morbidity of schizophrenia, anxiety disorder, and ID in patients with *LINS1* mutation. Our observations expand the clinical phenotype spectrum in patients with *LINS1* mutations.

Human LINS1 protein comprises 757 amino acids and is expressed in various tissues, including adult testis, prostate, spleen, thymus, skeletal muscle, fetal kidney, and brain [6]. The physiological function of *LINS1* in human development is still unclear. Studies of *Lines* gene, the homolog of *LINS1* in Drosophila, showed that the *Lines* gene was involved in the development of several tissues and organs. The *Lines* gene was essential for tissue- and stage-specific Wnt signaling events in the cell-fate specification. Activation of Wnt signaling promoted the entry of the lin protein encoded by the *Lines* gene into nuclei [13]. Hence, it is likely that the human LINS1 protein might be involved in the WNT signaling pathway. WNT signaling is essential for embryonic development in all animals. There are several pathways in WNT signaling, including canonical and non-canonic pathways. Mutations of genes encoding the proteins involved in the WNT signaling are linked to several human diseases. WNT signaling pathway also plays an essential role in regulating neuronal connectivity in the nervous system. Aberrant WNT signaling pathway has been associated with ID [14,15] and psychiatric disorders such as autism [14,16], bipolar disorder [17,18], and schizophrenia [19,20]. The Gln92X mutation of *LINS1* identified in this study was predicted to generate a loss-of-function truncated LINS1 protein. We speculate that the loss-of-function mutant may interfere with the integral development of neurons in the brain and lead to ID and other developmental and neuropsychiatric conditions. Further studies are needed to elucidate the details of the pathogenesis.

Conflicts of interest

All the authors declare no conflict of interest.

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