

# A novel *de novo* hemizygous *ARHGEF9* mutation associated with severe intellectual disability and epilepsy: a case report

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## Abstract

*ARHGEF9* encodes collybistin, a brain-specific guanosine diphosphate-guanosine-5'-triphosphate exchange factor that plays an important role in clustering of gephyrin and  $\gamma$ -aminobutyric acid type A receptors in the postsynaptic membrane. Overwhelming evidence suggests that defects in this protein can cause X-linked intellectual disability, which comprises a series of clinical phenotypes, including autism spectrum disorder, behavior disorder, intellectual disability, and febrile seizures. Here, we report a boy with clinical symptoms of severe intellectual disability, epilepsy, and developmental delay and regression. Trio exome sequencing (*trio*-clinical exome sequencing) identified a novel hemizygous deletion, c.656\_c.669delACTTCTTTGAGGCC (p. His219Leufs\*9), in exon 5 of *ARHGEF9*. This variant was not reported in either the Genome Aggregation Database or our database of 309 patients with neurodevelopmental disorders. Oxcarbazepine and levetiracetam reduced the frequency of the patient's epileptic seizures to a certain extent, but psychomotor developmental delay and developmental regression became more obvious with age. This case study seeks to report a *de novo* loss-of-function mutation of *ARHGEF9*, aiming to emphasize the genetic diagnosis of X-linked intellectual disability and further improve knowledge of the ethnic distribution of *ARHGEF9* mutations.

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## Keywords

ARHGEF9, epilepsy, X-linked intellectual disability, protein truncation, loss-of-function mutation, exome sequencing

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## Background

Intellectual disability (ID) is a developmental brain disorder commonly characterized by mental retardation and adaptive behavior. Approximately 1% of people worldwide suffer from various degrees of ID.<sup>1</sup> In particular, according to a consensus estimate, males are affected by ID approximately 30% to 40% more often than females, and a major portion of patients are likely to have a clear X-linked segregation.<sup>2</sup> Although only approximately 5% of the entire complement of human genes is located on the X chromosome, 15% of X-linked genes are identified as ID risk genes.<sup>3</sup>

Previous studies have shown that X-linked intellectual disability (XLID) is highly heterogeneous in both genetic and clinical aspects. XLID can usually be divided into two groups: syndromic ID, with physical features and comorbid illnesses, and nonsyndromic ID, without obvious comorbidities.<sup>4,5</sup> Although next-generation sequencing (NGS) and diagnostic techniques have improved, currently, the majority of ID genes are still uncharacterized or not well-described. Therefore, more specific research is needed to clarify the mutations causing XLID. In recent years, a series of large-scale clinical cohort studies has been conducted. Through high-throughput and high-resolution microarrays and NGS technology, 150 XLID genes have been identified. Moreover, it has been posited that most established XLID genes play a crucial

role in brain development, particularly in synaptic function.<sup>5,6</sup>

*ARHGEF9* is one such gene on chromosome X q11. It acts as a guanine nucleotide exchange factor (GEF) for CDC42. Defects in this gene can cause epilepsy, ID (moderate to severe), developmental delay, autism spectrum disorder, and several other types of disability.<sup>7</sup> Currently, 27 pathogenic variants have been recorded, corresponding to 9 female and 18 male patients.<sup>5,7-19</sup> Six copy number variants (CNVs) and one missense and two loss-of-function variants have been identified in female patients. All of these female patients have estimated X-chromosome inactivation (XCI). Compared with affected females, most male patients present severe clinical symptoms. In this case, we report a novel *de novo* loss-of-function (LoF) mutation identified in a male patient presenting with ID, developmental delay, and epilepsy. During the course of treatment, developmental regression was also noted. This case report describes a *de novo* single nucleotide variant (SNV) that connects the *ARHGEF9* gene with developmental regression.

## Materials and methods

### Clinical data

A Tibetan family was recruited at the Rehabilitation Medicine Center of the West China Second University Hospital. Clinical data were obtained using a standard assessment of neurodevelopmental

disorders, including basic personal information, birth history, family history, physical examination (head circumference, muscle tone, facial appearance), system development (motor development, language development, autism, intelligence, sleep disorders, social interaction, seizures), and auxiliary examinations (head magnetic resonance imaging (MRI), electroencephalography, biochemical and metabolic indicators). All results were evaluated by two qualified pediatric neurologists. Signed informed consent was obtained from the patient's parents, and the study was approved before the project began by the Ethical Committee of West China Second University Hospital of Sichuan University (K2017021). The reporting of this case conformed to CARE guidelines.<sup>20</sup>

## NGS

NGS was performed by a commercial vendor (Novogene, Tianjin, China) using a HiSeq 2000 platform (Illumina, San Diego, CA, USA). Sequencing libraries were generated using a SureSelect Human All Exon kit V6 (Agilent Technologies, Santa Clara, CA, USA). Processed reads were aligned to the human reference genome (GRCh38) using BWA v01.22 (<https://github.com/lh3/bwa>).<sup>21</sup> SNVs and insertion–deletion mutants were detected by SAMtools v1.0 (<https://sourceforge.net/projects/samtools/files/samtools/1.0>).<sup>22</sup> Functional annotation was performed by ANOVAR (<https://annovar.openbioinformatics.org/en/latest/user-guide/download>) and compared with the following databases: 1000 Genomes, dbSNP, Combined Annotation Dependent Depletion (CADD), Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), and Human Gene Mutation Database (HGMD).<sup>23</sup> Mutations were removed if the minor allele frequency of the mutation was

>0.1% in more than one of the frequency databases, including the 1000 Genomes data (1000g\_all), ExAC\_all, and gnomAD (gnomAD\_ALL and gnomAD\_EAS).<sup>24</sup> *De novo* mutations were searched according to family-based mutation information using SAMtools. Sanger sequencing was used to validate the identified potential disease-causing variant.

## Electroencephalography

Electroencephalography was used as a neurodiagnostic test to record the electrical activity of the brain.<sup>25</sup> Routine, scalp, awake, and interictal electroencephalogram (EEG) recordings were performed on the patient. Cap electrodes were applied with collodion, according to the 10–20 system, with linked mandibular references. The data were obtained on 16-channel Grass and Medtronic electroencephalography instruments (Shanghai, China). Common average referential, longitudinal, and transverse bipolar montages were used in all examinations. EEG recordings were filtered with 1 Hz high-pass, 35 Hz low-pass, and 60 Hz notch filters at a paper speed of 30 mm/s.

## Case presentation

The Rehabilitation Medicine Center of the West China Second University Hospital has collected data on pediatric patients with neurodevelopmental disorders to better understand genetic neurodevelopmental conditions. Whole exome sequencing and CNV microarray technologies were used to establish a database of genetic samples from families with one proband and unaffected parents.

This male patient was the first child of healthy parents with no relevant family history. He was born at full term with spontaneous delivery after an uneventful pregnancy. His weight and length were

both normal at birth. At 1 year of age, he suddenly presented with several episodes of neurological distress characterized by blank staring and no response to communication but without losing bowel or bladder control. The seizure episodes, which occurred once or twice per month, lasted 1 to 2 minutes each, and the patient would exhibit a period of lethargy. On admission to our hospital, the outpatient doctor prescribed levetiracetam. The seizures continued, but the frequency of seizures decreased to once every 1 to 2 months. At 3 years of age, the patient began to exhibit more frequent seizures, at 2 to 4 times per month. Oxcarbazepine and levetiracetam biotherapy led to a decrease in the seizure frequency to once per month. At the age of 3 years and 1 month, the patient's weight was 11.9 kg (less than the fifth percentile), and his height was 93 cm (25th–50th percentile). The patient showed psychomotor developmental delays. However, he was able to sit and stand independently at 1 year of age and even walk alone at 23 months. Developmental regression was observed because of the frequent seizures. At the age of 3 years, he was suddenly unable to sit or stand without support. Currently, the patient cannot speak any words. Brain MRI did not show any morphological abnormality. Because the patient is young and lives in Tibet, he cannot attend the clinic regularly.

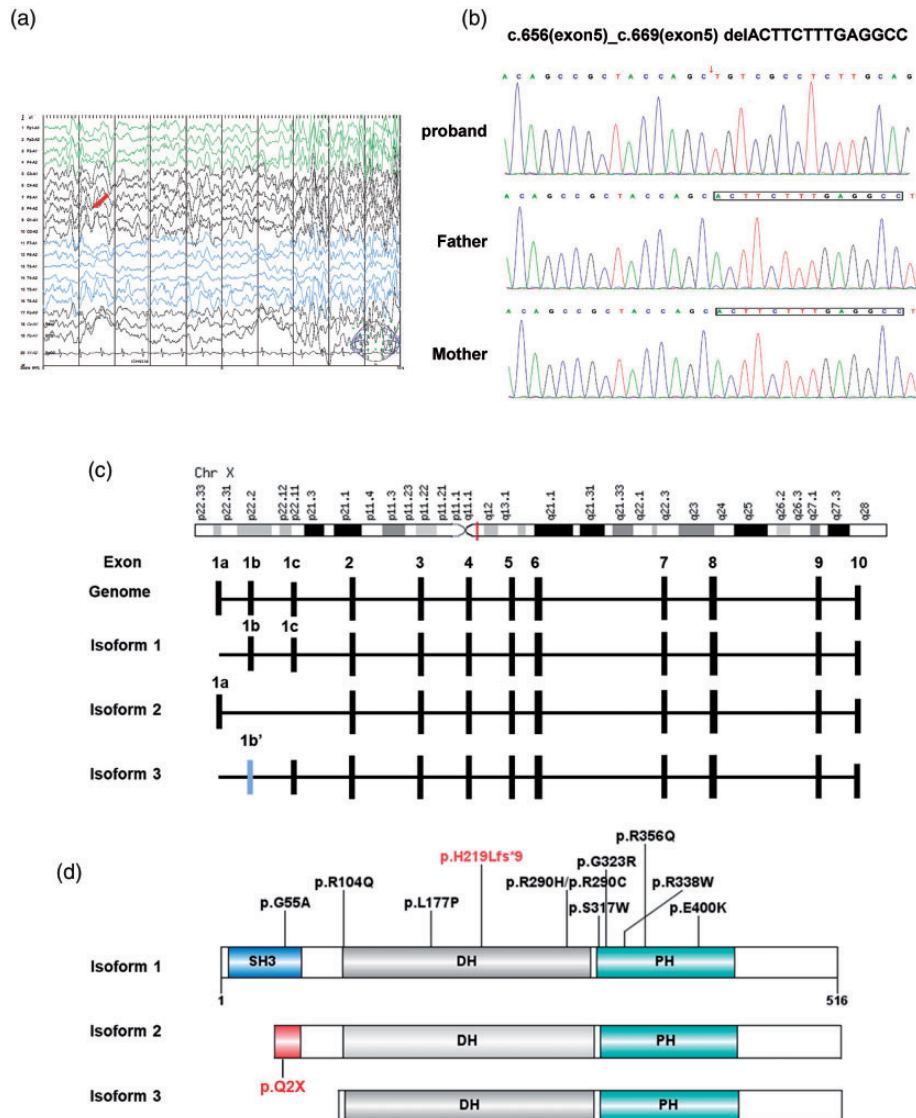
## Results

EEG results commonly provide strong evidence for underlying focal or diffuse cerebral dysfunction. This patient was instructed to remain relaxed during the EEG recordings. Electroencephalographic abnormalities occurred in this case, with bursts of generalized slow waves and focal slowing (red arrow) (Figure 1a). This slow

wave activity was considered pathologic, indicating corresponding generalized cerebral dysfunction. No clinical seizures were observed during EEG recordings.

Because CNV microarrays cannot identify known or candidate pathogenic variations, whole exome sequencing was performed and subsequently used to predict two candidate genes (*ARHGEF9* and *SRPX2*) that were X-linked, consistent with the clinical pattern of X-linked recessive inheritance. *SRPX2* has been associated with XLID, but evidence supporting a role of *SRPX2* in epilepsy and speech and cognitive defects is still questionable.<sup>26</sup> The missense *SRPX2* variant c.257G>A/p.R86H was observed with an allele frequency of 0.0035 in the gnomAD database and was considered as a single nucleotide polymorphism.<sup>24</sup>

Furthermore, a *de novo* frameshift mutation located in exon 5 of *ARHGEF9* (p. His219Leu fs\*9) was identified. *ARHGEF9* has previously been reported to be an XLID gene, which is consistent with the phenotypic characteristics of the proband in this study. Table 1 summarizes all of the genetic findings and associated clinical phenotypes of patients with sequence or structural variants of *ARHGEF9*, including nine cases of CNVs and 18 cases of SNVs/insertion–deletion mutants. In this case, a deletion caused a frameshift mutation (p. His219Leu fs\*9) and was proposed to encode a premature stop codon, thus producing a truncated protein lacking two important functional domains. This *ARHGEF9* mutation identified by trio-clinical exome sequencing was confirmed by Sanger sequencing in the proband and his unaffected parents (Figure 1b). This deletion was only present in the proband and not either of his parents, indicating that this is a *de novo* protein-truncating mutation.



**Figure 1.** Identification of a de novo mutation in *ARHGEF9*. (a) Electroencephalogram (EEG) of the proband in this study. The EEG recording was filtered with 1 Hz high-pass, 35 Hz low-pass, and 60 Hz notch filters at a paper speed of 30 mm/s. The red arrow indicates the location of slow waves. (b) Sanger sequencing of the *ARHGEF9* gene mutation. DNA sequence traces of *ARHGEF9* from the proband indicated a p.H219Lfs\*9 variant. (c) *ARHGEF9* is located on X chromosome (q11.1) and composed of 11 exons with three isoforms. (d) Schematic of *ARHGEF9* protein with Src homology 3 (SH3), Dbl homology (DH), and pleckstrin homology (PH) domains. All reported missense and frameshift variants are indicated. The mutation identified in this proband was predicted to cause a truncated protein (red).

**Table 1.** Genetic findings and phenotypic features of ARHGEF9 variants.

Variant	Sex	Inheritance	Symptoms	ID	MRI	Epilepsy	EEG findings	AEDs	Response to AEDs	Ref
<b>CNV</b>										
#1	F	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	VPA, PHT, PGB	Improved	(Marco et al. 2009) <sup>7</sup>
#2	F	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	CZP, VPA	Improved	(Kalscheuer et al. 2009) <sup>9</sup>
#3	F	De novo	DD	Moderate	NR	No	N/A	N/A	N/A	(Alber et al. 2017) <sup>13</sup>
#4	M	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	PB, CBZ, CLB, TPM, OXC, LEV	Controlled	(Lesca et al. 2011) <sup>10</sup>
#5	M	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	VPA	Controlled	(Shimijima et al. 2011) <sup>5</sup>
#6	M	De novo	DD, SZ	Severe	NR	Yes	Abnormal	VPA	Controlled	(Machado et al. 2016) <sup>11</sup>
#7	F	De novo	DD	Mild	NR	No	N/A	N/A	N/A	(Bhat et al. 2016) <sup>14</sup>
#8	F	De novo	DD	Moderate	Normal	No	N/A	N/A	N/A	(Alber et al. 2017) <sup>13</sup>
#9	F	De novo	DD, SZ	Moderate	NR	Yes	Normal	NR	Seizure free	(Alber et al. 2017) <sup>13</sup>
<b>Frameshift and Nonsense</b>										
#10	M	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	LEX, OXC	Improved	This study
#11	M	De novo	DD, SZ	Severe	NR	Yes	Abnormal	VPA, LEX	N/A	(Yao et al. 2020) <sup>17</sup>
#12	M	Maternal	DD, SZ	Severe	Abnormal	Yes	Abnormal	Multiple AEDs	Refractory	(Shimijima et al. 2011) <sup>5</sup>
#13	M	De novo	SZ	Severe	Abnormal	Yes	Abnormal	VPA, LEX, CZP	Refractory	(Ghesh et al. 2019) <sup>18</sup>
#14	F	Paternal	DD	Moderate	Normal	Yes	Normal	N/A	N/A	(Ghesh et al. 2019) <sup>18</sup>
#15	F	De novo	DD, SZ	Moderate	Normal	Yes	Abnormal	VPA, LEX	Partially controlled	(Ghesh et al. 2019) <sup>18</sup>
<b>Missense</b>										
#16	M	De novo	DD, SZ	Severe	Abnormal	Yes	NR	PB, LTG	Refractory	(Harvey et al. 2004) <sup>8</sup>
#17	M	De novo	DD, SZ	Severe	Abnormal	Yes	Abnormal	Multiple AEDs	Refractory	(Alber et al. 2017) <sup>13</sup>
#18	M	De novo	DD, SZ	Severe	Normal	Yes	Abnormal	VPA, LEV, LTG	Improved	(Alber et al. 2017) <sup>13</sup>
#19	F	De novo	DD, SZ	Moderate	N/A	Yes	N/A	N/A	N/A	(Scala et al. 2021) <sup>19</sup>
#20	M	Maternal	DD, SZ	Severe	N/A	Yes	Abnormal	Multiple AEDs	Refractory	(J. Y. Wang et al. 2018) <sup>12</sup>
#21	M	De novo	DD, SZ	Moderate	Abnormal	Yes	Abnormal	Multiple AEDs	Refractory	(Alber et al. 2017) <sup>13</sup>
#22	M	Maternal	DD, SZ	Severe	Normal	Yes	Normal	VPA	Refractory	(Zhang et al. 2020) <sup>17</sup>
#23	M	Maternal	SZ	Severe	Abnormal	Yes	Abnormal	VPA, CBZ, CLB	Improved	(Alber et al. 2017) <sup>13</sup>

(continued)



**Table 1.** Continued.

Variant	Sex	Inheritance	Symptoms	ID	MRI	Epilepsy	EEG findings	AEDs	Response to AEDs	Ref
#24 c.967G>A (p. G323R)	M	Maternal	DD, SZ	Severe	Abnormal	Yes	Abnormal	VPA	Seizure free	(Klein et al. 2017) <sup>33</sup>
#25 c.1012C>T (p. R338W)	M	Maternal	SZ	Moderate	N/A	Yes	N/A	N/A	N/A	(Long et al. 2015) <sup>15</sup>
#26 c.1067G>A (p. R356Q)	M	Maternal	-	Mild	N/A	No	N/A	N/A	N/A	(Alber et al. 2017) <sup>13</sup>
#27 c.1070G>T (p. R357I)	M	Maternal	DD, SZ	Mild	Normal	Yes	Normal	N/A	N/A	(Zhang et al. 2020) <sup>17</sup>
#28 c.1198G>A (p. E400K)	M	De novo	DD	Moderate	Normal	No	N/A	N/A	N/A	(de Ligt et al. 2012) <sup>16</sup>

VPA, valproic acid; PH-T, phenytoin; PGB, pregabalin; CLB, clobazam; CBZ, carbamazepine; PB, phenobarbitone; TPM, topiramate; LEV, levobupivacaine; OXC, oxcarbazepine; CZP, clobazepam; LTG, lamotrigine; N/A, not applicable; NR, not reported; ID, intellectual disability; MRI, magnetic resonance imaging; EEG, electroencephalogram; AED, anti-epileptic drug; DD, developmental delay; SZ, seizure; CNV, copy number variant; LEX, levetiracetam; M, male; F, female.

## Discussion

*ARHGEF9*, which encodes collybistin (also known as hPEM2 in humans), can modulate cognitive function through its structural and functional activities.<sup>27</sup> Collybistin serves as a synaptic Rho GEF (for Rho/Rac/Cdc42-like GTPases) because it contains a Dbl homology (DH) domain. Rho GEFs have been shown to activate small GTPases of the Rho family by rapidly catalyzing the dissociation of GDP, allowing binding of GTP to induce an active state.<sup>28</sup> Additionally, a DH domain composed of approximately 180 amino acids is conserved among all Rho GEF family members and is responsible for the interactions and activation of Rho GTPase. The DH domain of collybistin activates Cdc42 but does not activate Rac1 or RhoA.<sup>29</sup> This domain is also believed to be involved in an interaction with gephyrin, which is an extensively studied scaffold protein responsible for anchoring, clustering, and stabilizing glycine and  $\gamma$ -aminobutyric acid type A receptors.<sup>30</sup> Moreover, it is critical for inhibitory synapse formation and function. However, the role of DH domain activity on Cdc42 in gephyrin clustering is unclear. Experiments in mice have shown that gephyrin and  $\gamma$ -aminobutyric acid type A receptor clustering were not affected by hippocampal deletion of Cdc42.<sup>29</sup> In our study, this frameshift mutation deleted 14 nucleotides in the center of the DH domain, leading to a functionally defective isoform with a disrupted DH domain and total loss of the pleckstrin homology domain and the C terminus. To our knowledge, nine CNVs and 18 frameshift/misense mutations have currently been identified in patients with *ARHGEF9*-related disorders (Table 1). However, the genotype-phenotype relationship has not been well correlated.

Functionally-deficient *ARHGEF9* caused by chromosomal abnormalities has been

previously found in nine patients, including six female patients who suffered from complete or extreme skewed X inactivation in favor of the abnormal X chromosome, and their conditions were similar to those of hemizygous male patients.<sup>13</sup> However, despite chromosome-wide silencing, approximately 15% of X-linked genes escape X inactivation, thus some XLID genes can be partially expressed because of another inactive X chromosome.<sup>3,31</sup> To date, three male patients with inactivation of the *ARHGEF9* gene caused by chromosome abnormalities have been reported. The lengths of the missing fragments were 1290 kb, 737 kb, and 217 kb. These three fragment deletions were all located immediately below the centromere and included the entire *ARHGEF9* gene as well as a micro-RNA gene (mIR1468). A 1290-kb Xq11.1 microdeletion disrupting *ARHGEF9* was found in a 6-year-old male patient with severe ID, epilepsy (onset: 5 months), developmental delay, macrosomia, and mild dysmorphism. The patient was hyperactive with attention deficit disorder, and his brain MRI results were normal.<sup>10</sup> The second reported microdeletion mutation, a 737-kb Xq11.1 deletion containing *ARHGEF9*, was identified by Shimojima et al. in a 5-year-old boy who also suffered from severe ID, epilepsy (onset: 24 months), development delay, and macrosomia. Brain MRI (Table 1) showed a relatively hypoplastic frontal lobe, and dysmorphic features were not reported in this patient.<sup>5</sup> The third 217-kb deletion was reported by Machado et al. in an 18-year-old male patient with severe mental impairment, developmental delay, epilepsy, and no dysmorphic features.<sup>11</sup> Similarly, the most significant phenotypes in the present patient were developmental delay and epilepsy, which are consistent with the above three patients.

Affected patients with *ARHGEF9* variants include nine female patients exhibiting six chromosomal abnormalities, two LoF

variants, and one missense variant. Seven of these patients presented with moderate ID and a less severe phenotype. Most affected females present skewed XCI patterns in the peripheral blood. Moreover, the mothers of the affected male patients were all healthy, indicating that the random skewing of X inactivation protects females from X-linked recessive disorders. Although Ghesh et al. reported two female patients with random XCI patterns, the XCI patterns in the peripheral blood may be different from those in other tissues, including the brain.<sup>18,32</sup>

According to the gnomAD database, the probability of being loss-of-function intolerant (pLI) score of *ARHGEF9* is 1, reflecting that this gene is extremely LoF intolerant. In total, only five LoF variants of *ARHGEF9* have been reported in the literature. Shimojima et al. identified the first nonsense variant in a cohort study.<sup>5</sup> Twenty-three male patients with severe ID and epilepsy were recruited, and their genotypes were verified by Sanger sequencing. A p.Q2 variant, which was located in the first exon, was identified in a 5.5-year-old boy with severe ID, epilepsy (onset: 20 months), and developmental delay.<sup>5</sup> Exon 1a encodes a long N terminus (MQWIRGGSGM) that is only present in isoform 2 of *ARHGEF9*. However, isoform 1 and isoform 3 begin with a shorter sequence (MTL) encoded by exon 1b (Figure 1c), indicating that p.Q2 only affects the expression of isoform 2 and does not affect the other two isoforms. Because this mutation occurred in the second codon of the variants, it represents total LoF of the encoding protein (Figure 1d). Another protein truncation mutation was recently reported by Yao et al.<sup>17</sup> It was a splice variant that could cause a truncated protein, which was shown by a transcriptional experiment.<sup>17</sup> Ghesh et al. recently reported three novel LoF variants (p.K352/E7 skipping,



p.R289\*, and p.W300\*), which is the first report of affected females with random XCI patterns, even when taking missense variants into account.<sup>18</sup> The present study reported a novel nonsense variant in a male patient. Similar to other males with LoF variants of *ARHGEF9*, severe ID and epilepsy were observed. Moreover, this report provides further evidence of developmental regression in affected patients with pathogenic *ARHGEF9* variants. However, as this patient could not visit the clinic often, we could not determine the precise time that developmental regression occurred.

## Conclusion

We report a patient with XLID with severe mental retardation, developmental regression, and epilepsy. Sequencing analysis identified a novel *ARHGEF9* mutation (c.656\_c.669delACTTCTTTGAGGCC). This deletion is a frameshift mutation (p. His219Leu fs\*9) and is proposed to encode a premature stop codon, thus producing a truncated protein. The lack of pleckstrin homology and DH domains led to LoF of collybistin. More importantly, this is one of the few clinical cases in which *ARHGEF9* has been associated with developmental regression, and thus it is crucial for extending the knowledge of XLID and NGS analysis.

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## Availability of data and materials

The annotated mutations of this patient and the raw NGS data are available upon request from the corresponding author.

## Author contributions

T. Q. conceived and designed the experiments and analyzed data. Q. D. and Q. W. wrote the manuscript.

## Ethics approval and consent to participate

The study was approved by the Ethical Committee of West China Second University Hospital of Sichuan University (K2017021). Written informed consent was obtained from the parents of this patient at the time of diagnosis.


## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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