

## ORIGINAL ARTICLE

# A novel variant in the HX repeat motif of *ATN1* in a Chinese patient with CHEDDA syndrome and literature review

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## Abstract

**Background:** CHEDDA syndrome is a rare neurodevelopmental syndrome caused by heterozygous missense or indel variants in the HX repeat motif of *ATN1* gene. To date, CHEDDA has been identified in a few ethnic groups, and only 17 patients have been reported in literature, and no case has been reported in any country or region in Asia.

**Methods:** Trio-exome sequencing (Trio-ES) examination was conducted in a Chinese girl with global developmental delay and in her parents. Sanger sequencing was performed to confirm the candidate variant.

**Results:** This patient presented with mental and motor developmental delay, speech delay, and mild dysmorphic facial features, and had no epilepsy and visual impairment. Brain MRI did not show obvious structural abnormality. Through ES we identified a novel and de novo variant, c.3176\_c.3177insGCACCT (p.Ser1059\_His1060insHisLeu), within the HX motif of *ATN1*. No other pathogenic variant in another gene was found to support an alternative clinical and molecular diagnosis.

**Conclusions:** This is the first described case of CHEDDA from China. Together with the available literature data, we found that either disruption of HX motif or alteration of the HX repeat number would lead to *ATN1*-associated CHEDDA. We also noted that CHEDDA is a clinical heterogenous syndrome, and patients carrying the same or similar variant might have different clinical manifestations and prognosis.

## KEYWORDS

*ATN1*, CHEDDA, developmental delay, HX repeat motif

Sukun Luo, Yanqiu Hu and Ping Xiong contributed equally to this study.

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## 1 | INTRODUCTION

CHEDDA (MIM: 618494) is a rare neurodevelopmental disorder which is characterized by congenital hypotonia, epilepsy, developmental delay, and digit abnormalities. It is caused by de novo missense, indel (insertion or deletion) variants within a conserved and repeated HX motif of *ATN1* encompassing amino acid residues 1049–1065, where H is a histidine and X represents any amino acid. In 2007, Mosca et al. reported a female proband with multiple congenital anomalies such as dysmorphic facial features, cleft palate, axial hypotonia, peripheral hypertonia, polymicrogyria, and cerebellar vermis hypoplasia (Mosca et al., 2007). The described features were thought to be unique and likely represent a new syndrome despite lacking molecular genetics evidences. Afterwards this patient was included in a neurocognitive syndrome cohort reported by Palmer et al. (2019), in which all patients harbored a de novo variant in the HX motif of *ATN1* gene (MIM: 607462) (Palmer et al., 2019). The new syndrome was named as CHEDDA to distinguish it from another neurodegenerative disorder called dentatorubropallidoluysian atrophy (DRPLA) (MIM: 125370), and that is caused by the abnormal expansion of CAG repeats in exon 5 of *ATN1* (Carroll et al., 2018).

Unlike DRPLA, CHEDDA syndrome is a non-progressive neurodevelopmental disorder, usually onset at birth, due to heterozygous disruption of the HX repeat in exon 7 of *ATN1*. CHEDDA syndrome was characterized with severity-varying universal symptoms of global developmental delay, infantile hypotonia, and dysmorphic facial features, with or without epilepsy, congenital malformations of brain, heart, and other organs as well as impairments of vision and hearing. Due to the small number of reported cases, the genotype–phenotype association of CHEDDA is currently unclear. Palmer et al. speculated that arrangement changing of histidine residues in the HX repeat may play a key role in structural and functional changing of *ATN1* (Palmer et al., 2019).

Here we described a Chinese CHEDDA patient, a 4-year-and-6-month-old girl who had motor developmental delay with unsteady gait, absent speech, and mild facial abnormalities. Using exome sequencing (ES),

we identified a novel and de novo insertion variant, NM\_001007026.2:c.3176\_c.3177insGCACCT (p.Ser1059\_His1060insHisLeu), in *ATN1*. To our knowledge, this is the first report of CHEDDA case in Chinese population.

## 2 | METHOD

### 2.1 | Ethical compliance

This study was approved by the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital) ethics committee and informed consent was obtained from parents of the patient.

### 2.2 | Sample preparation

Genomic DNA was extracted from 2 ml whole blood according to the protocol of the Omega DNA Mini Kit. A NanoDrop™ spectrophotometer was used for quality control of DNA purity and concentration.

### 2.3 | Exome sequencing

Genetic tests were performed by the Chigene corporation (Beijing, China). Genomic DNA was sheared by sonication and exome sequences were enriched by IDT xGen® Exome Researcher Panel v1.0, according to the manufacturer's protocol. DNA libraries were sequenced on Illumina HiseqXTen with a method of pair-end 150 bp reads. Raw image files were processed by the BclToFastq (Illumina) for base calling and raw data production. Low quality variants were filtered out using the quality score  $\geq 20$  (Q20) standard. The sequencing reads were then mapped to the human reference genome (hg19/GRCh37) with BWA. Single-nucleotide variations (SNVs) and Indels were scored by GATK 3.8. All short variants were annotated with databases including 1000 genomes, dbSNP, Exac, GnomAD, ClinVar, HGMD, and OMIM. Impacts of variants on protein functions were predicted using software packages Provean, SIFT, Polyphen2\_HDIV,

Polyphen2\_HVAR, Mutationtaster, M-CAP, and REVEL. The pathogenicity of candidate variants was evaluated based on phenotype, the MAF (minor allele frequency), conservation, and structural change of protein, and the inheritance pattern. The pathogenicity of identified variants was assessed in accordance with the American College of Medical Genetics (ACMG) clinical practicing guidelines (Richards et al., 2015). Candidate variants were confirmed in the patient and her parents using Sanger sequencing. The reference sequence used for *ATN1* was NM\_001007026.2.

### 3 | RESULT

#### 3.1 | Case report

The patient (currently 4.5 years old) was the first child of a healthy non-consanguineous Chinese couple without any neurodevelopmental disorder. She was delivered at 41 weeks of gestation by cesarean. Birth weight was 3.5 kg and born length was 50 cm. Her gross motor development was abnormal, with rolling over at 6 months, sitting on her own at 10 months, and starting to walk at 18 months. She had no feeding difficulties or epilepsy, except for one febrile convulsion at 12 months of age. At 21 months, she was brought to hospital because she walked unsteadily and had severe speech delay. It was noted that the patient had mild abnormal facial features, including hypertelorism, up-slanting palpebral fissures, frontal bossing, high hairline, and bulbous nasal tip. In addition, she had dysplasia of laryngeal cartilages, obstructive apnea, otitis media, and tootitis media with effusions. No visual and hearing problem was found.

Brain MRI and electroencephalogram (EEG) were performed and showed no remarkable change at the age of 21 months. By ES, we found a de novo variant, c.3176\_c.3177insGCACCT(p.Ser1059\_His1060insHisLeu) in the HX repeat region of *ATN1* (Figure 1a), annotated as “likely pathogenic” according to the ACMG (American College of Medical Genetics) guidelines. Except for this *ATN1* variant, genetic tests did not disclose any other pathogenic or likely pathogenic variant associated with phenotypes of the patient. Though the variant was not documented in the public databases or previous publication, it resulted in insertion of a histidine and leucine within the HX repeat motif of *ATN1*, which exerted a similar influence on the HX motif at amino acid level, compared with a previously reported variant c.3188\_3189dup (p.Leu1063\_His1064dup) (Figure 1c).

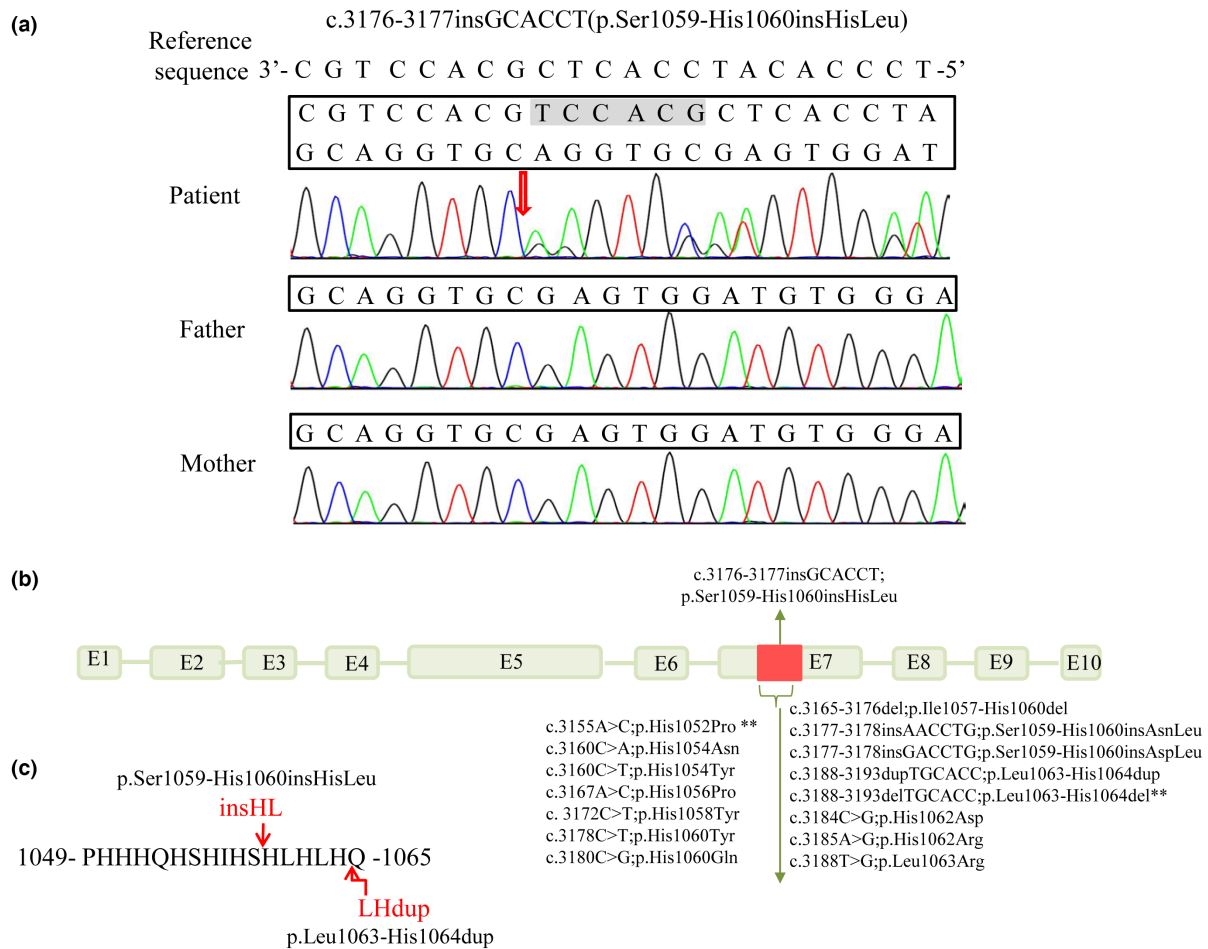
After the diagnosis was confirmed, the patient received regular and specialized training to improve her motor and speech ability. After 2 years of rehabilitation therapy, the

patient was able to walk independently, but had few improvements in the verbal and cognitive abilities. She had no clear words except “mama, papa” and yelled to get parents’ attention, and completely did not follow others’ instructions. Brain was re-examined and no remarkable change was found.

#### 3.2 | Literature review of patients with CHEDDA

Literature review of CHEDDA cases was conducted by searching for all cases published with the keywords “*ATN1*” and “CHEDDA”. The database included PubMed, Medline, ClinVar, and HGMD. By carefully verifying and avoiding the duplication of cases, we finally found 4 articles including 17 cases with CHEDDA and 15 variants, including 10 missense mutations and 5 indels (Figure 1b) (Hui et al., 2020; Mosca et al., 2007; Palmer et al., 2019, 2021). By comparing the clinical manifestations of all CHEDDA patients, we found that all patients presented infantile hypotonia, global developmental delay, and dysmorphic facial features. More than 80% of patients might have eye or vision abnormality, gastrointestinal malformation as well as anomalies of hand and feet. In addition, epilepsy, structural brain malformation, heart defect, hearing impairment, genitourinary and skeletal abnormality were also frequent among patients (Table 1). It was noteworthy that the insertion variant in our study, as well as three indel variants, c.3615\_3176del(p.Ile1057\_His1060del), c.3188\_3193del(p.Leu1063\_His1064del), and p.Leu1063\_His1064dup, reported in CHEDDA patients in 2021 (Palmer et al., 2021), did not interrupt the HX arrangement but change the length of HX repeats. Therefore, both the strict arrangement and length of HX motif might be critical for *ATN1* function. In addition, it was supposed an insertion of a histidine and another amino acid, compared with missense and other types of indel variants, may correlate with milder phenotype and better response to therapy (Palmer et al., 2021). It is true that our patient who carried an insertion of a histidine and leucine has milder motor developmental delay (walk unsteadily), however, she has profound cognitive and speech impairment, and early intervention had no obvious improvement. In brief, there is high heterogeneity even among patients with the similar or same variants.

In addition to these 15 variants annotated as “pathogenic” or “likely pathogenic”, a germline missense variant p.Ser1059Leu in ClinVar was identified in a patient presenting congenital hypotonia, epilepsy, developmental delay, and digital anomalies. Because this variant was classified as uncertain significance according to the ACMG guideline and the clinical and molecular diagnosis of this



**FIGURE 1** (a) Sanger sequencing of *ATN1* mutation in this family. (b) the locations of the *ATN1* missense or indel variants reported in individuals with CHEDDA. The red box represents the HX repeat motif, residing at the exon 7 of *ATN1* gene. Variant on the top is described in this cohort while variants on the bottom are previously reported in literatures. (c) the arrangement of HX repeat motif and location of p.Ser1059\_His1060insHisLeu and p.Leu1063\_His1064dup. All variants based on NM\_001007026.2.

patient was not confirmed, this case was not included in our summary.

## 4 | DISCUSSION

In this study, we reported the first case with CHEDDA from China. This patient had mild dysmorphic facial features, developmental delay and speech delay, and had no epilepsy, hearing, and visual concerns. Through ES, a de novo variant, c.3176\_c.3177insGCACCT (p.Ser1059\_His1060insHisLeu), was identified in the HX repeat region of *ATN1* gene. Together with the clinical features, the diagnosis of CHEDDA was made.

CHEDDA syndrome is a recently identified neurodevelopmental disorder characterized as severe global developmental delay including significant motor disability and impaired intellectual development, dysmorphic facial features, and variable congenital anomalies. Palmer et al. suggested global developmental delay, feeding difficulties,

and distinctive facial features as the core phenotypic features of CHEDDA (Palmer et al., 2021). The structural brain abnormalities have been documented to be associated with more severely impaired in neurodevelopment.

Protein *ATN1* belongs to the atrophin family proteins, and atrophin proteins are reported to be transcriptional co-repressors and involved in nuclear signaling (Wang & Tsai, 2008). *ATN1* is a small protein with 1191 amino acids, and is not essential during mouse development, however, converging evidence supports an important role in the control of brain and other organ system development. Polyglutamine expansion within glutamine-rich region (residues 484–502) of *ATN1* is associated with neurodegenerative disease, DRPLA, which presents dementia, choreoathetosis, myoclonus epilepsy, and ataxia (Shen et al., 2007; Wang & Tsai, 2008). This repeat expansion is thought to result in a toxic gain of function other than a simple loss of function of *ATN1* (Shen et al., 2007). Unlike DRPLA caused by the glutamine expansion in the glutamine-rich region in exon 5 of *ATN1*, CHEDDA



**TABLE 1** Phenotypic findings in CHEDDA patients in our cohort and previously published cohort

Phenotype	Our patient	#No. of patient (+/Total)	%
Prenatal abnormalities	–	8/18	44.4
Infantile hypotonia	+	18/18	100
Global developmental delay	+	18/18	100
Facial dysmorphism	+	18/18	100
Brain malformation	–	13/18	72.2
Seizure	–	10/18	55.6
Eye or vision abnormality	–	16/18	88.9
Hearing impairment	–	10/18	55.6
Heart defect	–	9/18	50.0
Gastrointestinal malformation	–	17/18	94.4
Genitourinary abnormality	–	9/18	50.0
Skeletal anomaly	–	13/18	72.2
Abnormality in hand and feet	–	16/18	88.9

Note: #: The patient in this study was included.

results from the indels and missense variants in the HX repeat motif in exon 7 encoding the residues 1049–1065. By reviewing literatures, we noted that the patients with an insertion of a histidine and another amino acid have milder phenotypes compared with those with a missense mutation, or a deletion of one amino acid, or insertion of two X (X is any amino acid except histidine) (Palmer et al., 2021). However, clinical heterogeneity was observed, even in individuals harboring the same or similar variants.

Previous studies suggested that the HX repeat of ATN1 might function as a motif for binding ions or other molecules in a pH-dependent manner and variants affecting the HX motif might alter its affinity for zinc ions (Janknecht et al., 1991; Palmer et al., 2019). Although the pathogenic mechanism underlying CHEDDA remains elusive, haploinsufficiency of ATN1 might not be the cause, because loss-of-function variants, including stop-gain, frameshift, and canonical splice variants, are found in healthy individuals in gnomAD database but not in patients with CHEDDA. In addition to *ATN1*, *RERE* (MIM: 605226), encoding another member of atrophin, is a pathogenic gene of neurodevelopmental delay (MIM: 616975). Compared with *ANT1* with 1911 aa, *RERE* is a larger protein (1566 aa) and its C-terminus is highly similar to that of *ATN1*. A variant, c.4313\_4318dup (p.Leu1438\_His1439dup), within the HX repeat motif of *RERE* has also been reported to be associated with a CHARGE-like multiple malformation syndrome (Jordan et al., 2018). Taken together, clinical features

resulted from the disruption of HX repeat motifs may be distinct from the neurodevelopmental conditions caused by other forms of genetic variants in same gene, and the underlying mechanisms are needed to be further studied.

In conclusion, we reported the first Chinese CHEDDA caused by a de novo insertion variant in the conserved HX repeats region of *ATN1*, and our study helps a better understanding of the *ATN1*-associated CHEDDA syndrome.

## AUTHOR CONTRIBUTIONS

**Xuelian He:** Study concepts; Data analysis/interpretation; Manuscript editing; Manuscript final version approval. **Cuiping Xiao:** Study concepts; Clinical information collection; Manuscript preparation; Manuscript final version approval. **Sukun Luo:** Study concepts; Study design; Data acquisition; Manuscript preparation. **Yanqiu Hu:** Study design; Literature research; Data acquisition; Data analysis/interpretation. **Hongmin Zhu:** Study design; Manuscript editing. **Li Tan:** Literature research. **Yufeng Huang:** Literature research. **Peiwei Zhao:** Clinical information collection; Manuscript preparation. **Ping Xiong:** Data acquisition; Data analysis/interpretation.

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## CONFLICT OF INTEREST

The author(s) declare that they have no competing interests.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

## ETHICS STATEMENT

This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of Wuhan Children's Hospital.

## INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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