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Association of *LONP1* gene with epilepsy and the sub-regional effect

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The *LONP1* gene encodes Lon protease, which is responsible for degrading damaged or misfolded proteins and binding mitochondrial DNA. Previously, *LONP1* variants have been identified in patients with cerebral, ocular, dental, auricular, and skeletal anomalies (CODAS syndrome) and mitochondrial diseases. Seizures were occasionally observed. However, the association between *LONP1* and epilepsy remains elusive. In this study, we performed trio-based whole-exome sequencing in a cohort of 450 patients with unexplained epilepsy and identified four pairs of compound heterozygous *LONP1* variants in four unrelated cases. All patients exhibited good responses to anti-seizure medications and demonstrated no developmental delay or intellectual disabilities. The variant allele frequencies observed in this study were absent or low in the general population and were significantly lower than those of benign variants. At least one variant in each biallelic pair affected hydrogen bonding and/or altered protein stability. The CODAS syndrome-associated variants were concentrated in the AAA+ module, especially the α domain. Four of the five mitochondrial disease-associated variants were located in the AAA + domain and the NTD^{5H} and NTD^{3H} subdomains. In contrast, each of the biallelic variants from the patients with pure epilepsy had one variant located in the linker domain, and the other variant located in the mitochondrial targeting sequence or P domain. This study suggested that *LONP1* gene is potentially a novel candidate gene for pure epilepsy. The phenotypic variation is associated with the sub-regional effects of variants.

Keywords *LONP1* gene, Epilepsy, Molecular sub-regional effect, Genotype-phenotype correlation

The *LONP1* gene (OMIM* 605490) encodes the ATP-dependent mitochondrial Lon protease. It is ubiquitously expressed, with the highest levels in the most metabolically active organs, including the brain (https://www.proteinatlas.org/ENSG00000196365-LONP1)[1]. The encoded protein is a multifaceted enzyme involved in the degradation of damaged or misfolded proteins, binding of mitochondrial mtDNA, and regulating several cellular processes within and outside mitochondria[2–4]. Homozygous deletion of *Lonp1* in mice is embryonic lethal with embryonic growth retardation[5].

In humans, biallelic variants in *LONP1* have been reported to cause cerebral, ocular, dental, auricular, and skeletal anomalies (CODAS syndrome, OMIM# 600373), a rare syndrome characterized by a distinctive constellation of features including developmental delay and multi-system developmental malformations[6,7]. *LONP1* variants also have been occasionally identified in patients with mitochondrial encephalopathy[8]. Affected individuals exhibited encephalopathy, pachygyria and microcephaly. Seizures were observed in a few cases[8,9]. However, there have been no reports of *LONP1* variants in patients with pure epilepsy, and the association between *LONP1* and epilepsy remains elusive.

In this study, trio-based whole exome sequencing (WES) was performed in a cohort of 450 patients with epilepsy without an acquired cause. Four compound heterozygous *LONP1* variants were identified in four unrelated individuals without neurodevelopmental disorders. These variants had no or low allele frequencies in the gnomAD database and affected hydrogen bonding and/or free energy stability of amino acids. To explore the possible mechanism underlying phenotype variations, we further systematically reviewed all the previously reported *LONP1* variants.

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Results

Identification of *LONP1* variants

Four pairs of compound heterozygous missense *LONP1* variations, including c.745G > C/p.Glu249Gln and c.152C > T/p.Pro51Leu; c.745G > C/p.Glu249Gln and c.2524 C > A/p.Leu842Met; c.686 C > T/p.Ala229Val and c.128 A > G/p.Gln43Arg; and c.2861 C > T/p.Ala954Val and c.686 C > T/p.Ala229Val, were identified in four sporadic cases with epilepsy (Fig. 1A, B; Table 1). The compound heterozygous variants originated from their asymptomatic parents, consistent with a classical recessive inheritance pattern.

Four variants c.152 C > T/p.Pro51Leu, c.2524 C > A/p.Leu842Met, c.128 A > G/p.Gln43Arg, and c.2861 C > T/p.Ala954Val presented no or extremely low frequencies (MAF < 0.00005) in the gnomAD database. Other variations (c.745G > C/p.Glu249Gln and c.686 C > T/p.Ala229Val) presented at low frequencies (MAF < 0.005) in the gnomAD-all population. Four variants, including c.128 A > G/p.Gln43Arg, c.152 C > T/p.Pro51Leu, c.2524 C > A/p.Leu842Met, and c.2861 C > T/p.Ala954Val, were absent in the controls of gnomAD East Asian population. None of these variants were observed in the homozygotes of the control population in gnomAD (Table 2).

All variants in this study, with the exception of p.Gln43Arg and p.Ala229Val, were predicted to be "damaging" by at least two in silico tools (http://varcards.biols.ac.cn/) (Supplementary Table 1).

The molecular effects of four pairs of compound heterozygous missense variants were analysed by using the Alphafold web tool for protein modelling and PyMOL software for visualization. One missense variant (p.Leu842Met) was predicted to alter hydrogen bonds with surrounding residues and the protein stability. Three variants (p. Pro51Leu, p. Ala229Val, and p. Ala954Val) altered hydrogen bonds with surrounding residues. One variant (p.Glu249Gln) did not change the hydrogen bonding with surrounding residues but altered the protein stability (Fig. 2C).

None of the affected individuals had pathogenic or likely pathogenic variants in genes known to be associated with epilepsy[10].

Clinical features of the patients with *LONP1* variants

The main clinical characteristics of the four cases with *LONP1* variants are summarized in Table 1. All patients were born to non-consanguineous parents after an uneventful pregnancy. Intellectual and motor development was normal for all patients, and no ocular, dental, auricular, or skeletal problems presented. Brain structure on MRI was normal in all the patients. The onset ages of seizures ranged from two to 19 years, and three of them (case 1, case 2, and case 4) had antecedent febrile seizures. All the patients showed infrequent seizures and achieved seizure-free with anti-seizure medications.

Case 1 presented with generalized tonic-clonic seizures (GTCS) as the age of two years. She became seizure free with levetiracetam (19.3 mg/kg/day) for 1 year.

Case 2 had five times GTCS within half a year. EEG showed sharp wave in bilateral post-temporal and occipital areas. She got seizure free for 2 years with treatment of levetiracetam (23 mg/kg/day).

Case 3 suffered from myoclonic seizures at age 15 years old. His seizures showed good response to topiramate (125 mg/day). Anti-seizure medication was withdrawal after seizure-free for 7 years. After 4 years, he had two (GTCS). Generalized polyspike-slow waves were observed in EEG recording (Fig. 3A) and topiramate (125 mg/day) was restarted. To date, he had been seizure-free for 3 years.

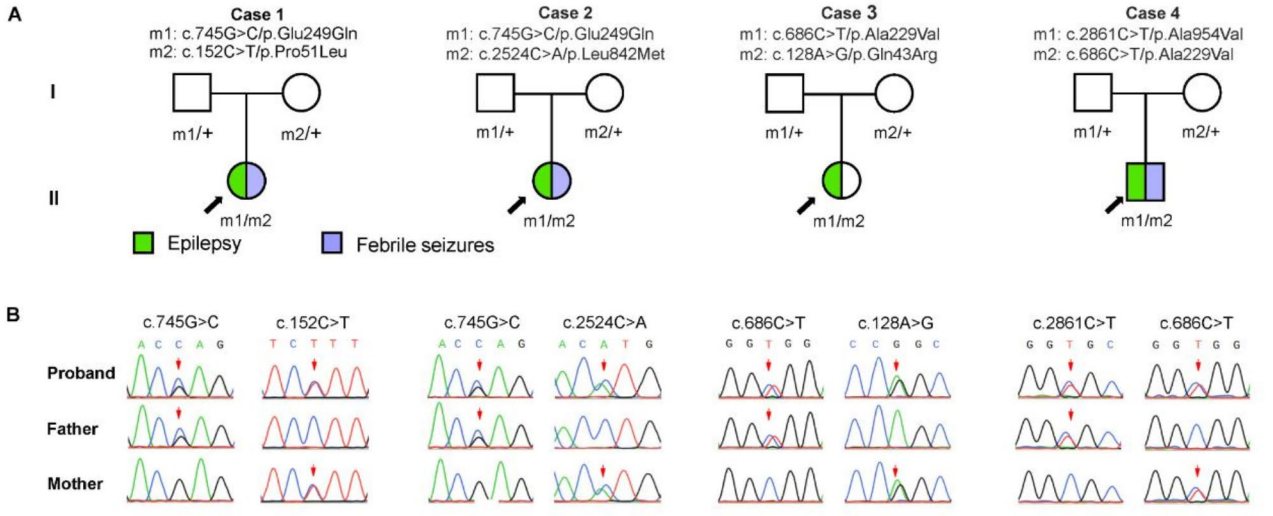

Fig. 1. Genetic data of cases with *LONP1* variants. **(A)** Pedigrees of the four cases with *LONP1* and their corresponding phenotypes. **(B)** DNA sequence chromatogram of the *LONP1* variants. The arrows indicate the positions of the variants.

Case No	Variant (NM_004793.4)	Sex	Present age	FS age (times)	aFs age	Seizure type and frequency	Seizure timing	Effective ASMs	EEG	Brain MRI	Development	Diagnosis	Seizure-free duration
1	c.745G > C/p.Glu249Gln c.152 C > T/p.Pro51Leu	F	3 yrs	1.5 yrs (once)	2 yrs	GTCS twice within 2 days	Diurnal	LEV	NA	Normal	Normal	EFS+	1 yr
2	c.745G > C/p.Glu249Gln c.2524 C > A/p.Leu842Met	F	9 yrs	3.5 yrs (once)	7 yrs	GTCS 5 times within half a yr	Diurnal and nocturnal	LEV	Sharp wave in bilateral post-temporal and occipital areas	Normal	Normal	EFS+	2 yrs
3	c.686 C > T/p.Ala229Val c.128 A > G/p.Gln43Arg	F	31 yrs	-	15 yrs	Myo 1–2 times/yr, GTCS twice in 4 yrs after AED withdraw	Diurnal	TPM	Generalized polyspike-slow waves	Normal	Normal	GE	3 yrs
4	c.2861 C > T/p.Ala954Val c.686 C > T/p.Ala229Val	M	29 yrs	5 yrs (once)	19 yrs	GTCS 1–2 times/yr	Nocturnal mostly	OXC	Sharp-slow waves in right anterior and middle temporal areas	Normal	Normal	EFS+	3 yrs

Table 1. Clinical features of individuals with *LONP1* variants. aFs, afebrile seizure; ASMs, anti-seizure medicine; EEG, electroencephalogram; EFS, epilepsy with febrile seizure plus; F, female; FS, febrile seizure; GE, generalized epilepsy; GTCS, generalized tonic–clonic seizure; LEV, levetiracetam; M, male; MRI, magnetic resonance imaging; Myo, myoclonic seizure; NA, not available; OXC, oxcarbazepine; TPM, topiramate; yr, year.

Variants NM_004793.4	Allele Count/ number in this study	Allele count/number in GnomAD-all population	Allele count/number in controls of gnomAD-all population	Allele count/number in gnomAD East Asian population	Allele count/number in control of gnomAD East Asian population	Homozygotes in the controls of gnomAD
c.745G > C/p.Glu249Gln	2/900 (2.22×10^{-3})	24/281 926 (8.51×10^{-5})	10/119 650 (8.36×10^{-5})	22/19 920 (1.10×10^{-3})	10/9 942 (1.01×10^{-3})	0
c.152 C > T/p.Pro51Leu	1/900 (1.11×10^{-3})	5/189 160 (2.64×10^{-5})	2/86 412 (2.31×10^{-5})	2/29 420 (6.80×10^{-5})	0/6 452 (0)	0
c.2524 C > A/p.Leu842Met	1/900 (1.11×10^{-3})	1/251 038 (3.98×10^{-6})	–/–	0/18 388 (0)	–/–	–
c.686 C > T/p.Ala229Val	2/900 (2.22×10^{-3})	145/281 716 (5.15×10^{-4})	74/120 028 (6.17×10^{-4})	133/19 912 (6.80×10^{-3})	68/9 950 (6.83×10^{-3})	0
c.128 A > G/p.Gln43Arg	1/900(1.11×10^{-3})	3/175 820 (1.71×10^{-5})	2/80 152 (2.50×10^{-5})	0/13 238 (0)	0/5 758 (0)	0
c.2861 C > T/p.Ala954Val	1/900 (1.11×10^{-3})	1/250 382 (3.99×10^{-6})	–/–	0/18 378 (0)	–/–	–

Table 2. Frequencies of *LONP1* variants identified in this study. gnomAD, Genome Aggregation Database.

Case 4 started to have GTCS 12-times 1–2/year. Interictal EEG indicated sharp-slow waves in the right anterior and middle temporal areas (Fig. 3B). He has been seizure-free with oxcarbazepine (900 mg/day) for 3 years.

Sub-regional effect of *LONP1* variants

Human Lon protease is composed of 959 amino acids, including a mitochondrial-targeting sequence (MTS) and three functional domains, i.e., an N-terminal domain (N domain), an ATPase domain (A domain), and a protease domain (P domain) (Fig. 2A)[11,12]. We systematically reviewed previously reported *LONP1* variants and their associated phenotypes. Variants identified in the present study and previously reported were depicted v in Fig. 2A.

Previously, a total of 14 biallelic variants were identified in 21 patients with CODAS syndrome[7,13–18]. These variants were concentrated in the AAA + module, especially the α domain. One monoallelic and three biallelic variants were identified in four cases with mitochondrial diseases[8,9,19,20]. Four of the five mitochondrial disease-associated variants were located in the AAA+ domain and the NTD^{5H} and NTD^{3H} subdomains. In contrast, each of the biallelic variants associated with pure epilepsy had one variant located in the linker domain, and the other variant located in the mitochondrial targeting sequence or P domain, suggesting a potential sub-regional effect (Fig. 2A).

Regarding epilepsy, one monoallelic and four biallelic variants were previously identified in five cases[7–9,13,19]. Detailed seizure information was available for the three patients (Supplementary Table 2). Among the three patients, the patient with variant located in the NTD^{5H} (p.Arg301Trp) showed neonatal refractory seizures[8]. One patient, who had compound heterozygous variant and one was missense variant located in P domain (p.Arg786Trp and p.Ser100Glnfs*46), showed toddler onset brief tonic seizures that were controlled with valproate[24]. Another patient with homozygous missense variant located in the start of the P domain (p.Pro761Leu) had drug-resistant epilepsy with multiple seizure types[9]. In contrast, patients in this study with variants located in the linker, MTS, and P domains presented with relatively late onset, mild epilepsies, and good response to anti-seizure medications. These results suggest a potential correlation between the location of *LONP1* variants and the severity of epilepsy.

Analysis of MAF difference between benign variants and epilepsy-associated variants

In this study, the frequencies of variants associated with pure epilepsy were significantly lower than those of the benign variants ($p = 0.00155$ in the gnomAD-all populations; $p = 0.01632$ in the gnomAD-control populations). Additionally, the frequencies of variants associated with the CODAS syndrome and mitochondrial disease were also significantly lower than those of the benign variants ($p = 6.657\times10^{-8}$ in the gnomAD-all populations, 6.435×10^{-7} in the gnomAD-control populations) (Fig. 2B).

Discussion

In this study, we identified *LONP1* compound heterozygous missense variants in four unrelated cases with infrequent seizures, without any developmental or intellectual disorders. These variants had no or low frequencies in the gnomAD database, which is significantly lower than those of benign variants. These variants were predicted to be damaging by silico tools, alter hydrogen bonds with surrounding residues, and/or change protein stability. Each of the biallelic variants was found to have one variant located in the linker domain, and the other variant located in the mitochondrial targeting sequence or P domain. In contrast, the CODAS syndrome-associated variants were densely located in the AAA + module, especially the α domain. Four of the five mitochondrial disease-associated variants were located in the AAA+ domain and the NTD^{5H} and NTD^{3H} subdomains. These results suggest that *LONP1* is potentially a candidate pathogenic gene of epilepsy with favourable outcomes and that the sub-molecular implications explain the phenotypic variation of *LONP1*.

The *LONP1* gene encodes Lon protease, which is conserved throughout evolution from bacteria to humans and responsible for regulating diverse aspects of mitochondrial biology, including proteostasis, electron transport chain activity, and mitochondrial transcription[2–4]. Homozygous deletion of Lonp1 in mice is embryonic lethal

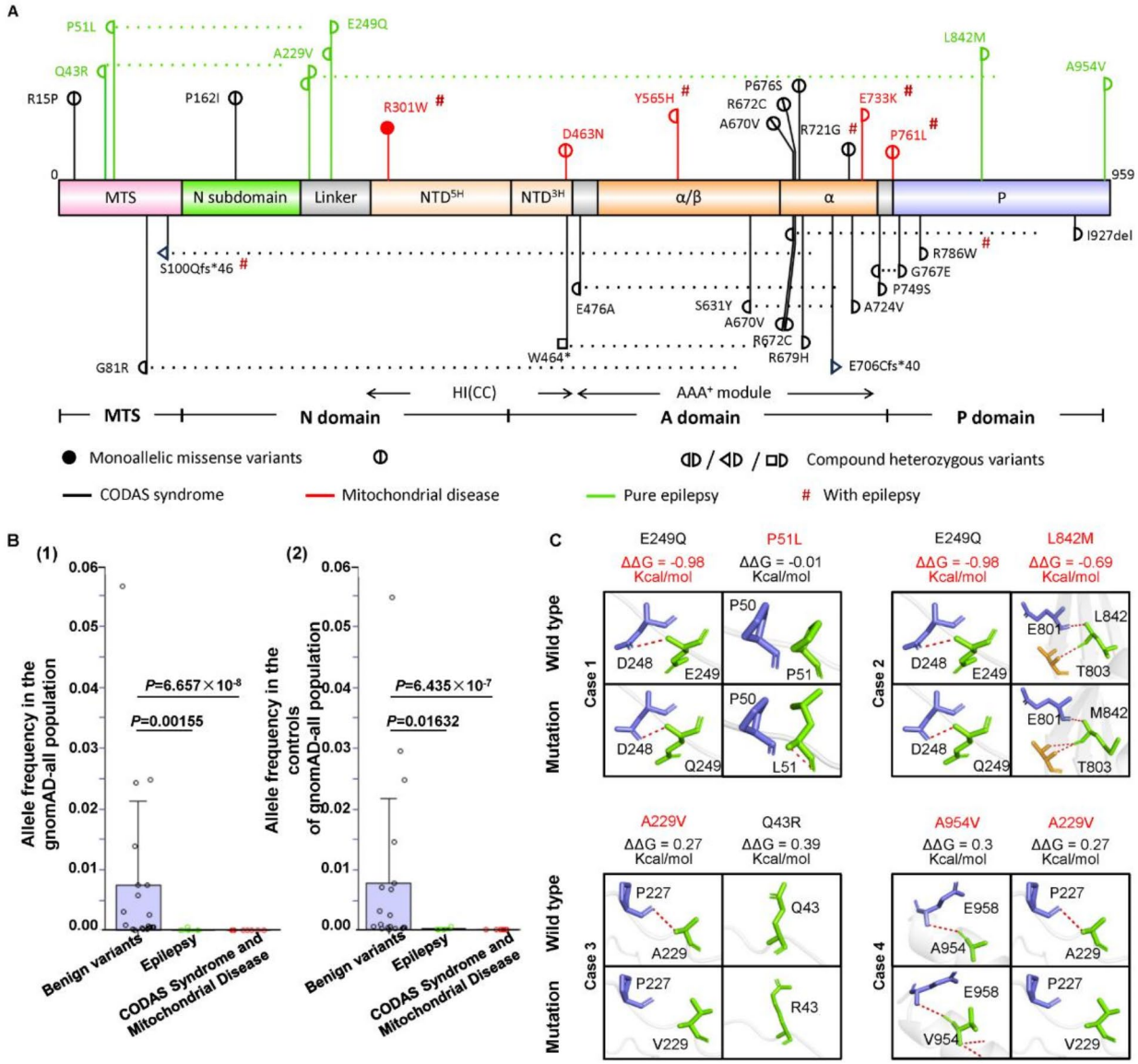

Fig. 2. Schematic illustration of Lon protease structure, statistical analysis of variant frequencies, and molecular effect of the six missense variants on Lon protease. **(A)** Schematic diagram of LONP1 and the localization of the variants of *LONP1* identified in previous reports and in this study. Two variants with connection of dotted lines represent a pair of biallelic variants. **(B)** Comparison of the frequencies of the epilepsy-associated variants, CODAS syndrome and mitochondrial disease-associated variants with that of benign missense variants retrieved from the ClinVar database. **(C)** Hydrogen bond changes and free energy stability changes (ΔΔG, Kcal/mol) value of the variants from the present study.

with poor development, underscoring Lon protease's importance in regulating mitochondrial function during organismal development[5]. Knockdown of Lonp1 in PV cells and CA1 neurons deteriorated status epilepticus-induced neuronal death, suggesting that Lon protease may contribute to the maintenance of neuronal viability[21]. Previous studies had identified *LONP1* variants in patients with CODAS syndrome and mitochondria diseases. Five cases accompanied by seizures, among which the detailed information of three cases were available[7–9,13,19]. The association between *LONP1* and epilepsy remains elusive due to the limited cases, variable phenotype, and insufficient clinical information. In the present study, we identified four biallelic variants in four patients with mild seizures without brain malformations or neurodevelopmental delay, suggesting that *LONP1* variants are potentially associated with epilepsy with favourable outcome.

The pLi score for *LONP1* is 0.999, indicating that it is highly intolerant to loss-of-function variants. The difference between the missense Z-score and the synonymous Z-score is 5.23, demonstrating that the protein is highly intolerant to missense variants. The variants in the present study exhibited no or low allele frequencies in the general population, with MAFs significantly lower than those of benign variants. At least one variant in

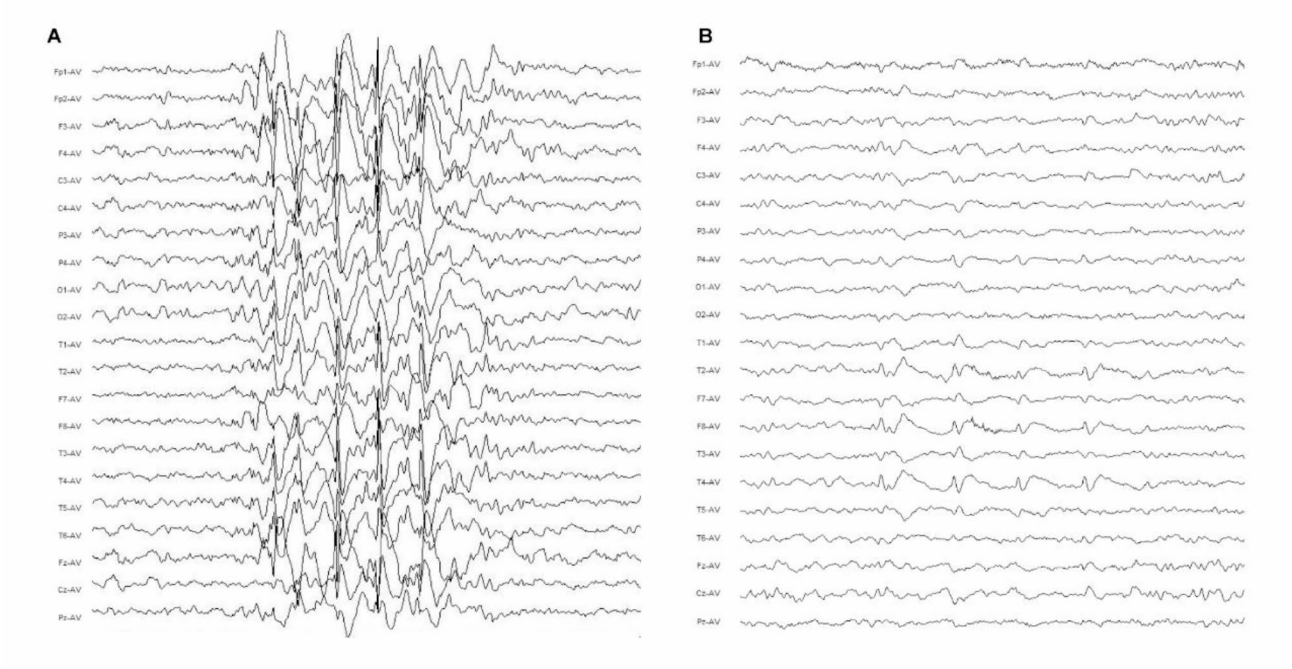

Fig. 3. Representative EEG recordings of the patients with *LONP1* variants. (A) Interictal EEG of case 3 indicated generalized polyspike-slow waves (obtained in one's twenties). (B) Interictal EEG of case 4 showed sharp-slow waves in the right anterior and middle temporal areas (recorded in one's twenties).

each biallelic pair affected hydrogen bonding and/or altered protein stability. Therefore, the combination of two variants inherited from asymptomatic parents was potentially damaging and resulted in epilepsy.

From the perspective of knockout (KO), homozygous KO mice showed embryonic lethality and growth retardation[5]. It is conceivable that biallelic truncating *LONP1* variants would result in early/embryonic death in humans, which is often less clinically identified. While heterozygous KO animals were phenotypically normal, mitochondrial size was larger and less circular, and basal oxygen consumption was lower, suggesting a dosage effect[22]. Previously, *LONP1* variants were identified in patients with severe CODAS syndrome and mitochondrial disease. The present study found that *LONP1* variants are also associated with mild epilepsy. In general, variants with severe damaging effects will result in severe diseases, whereas mildly damaged variants will lead to mild phenotypes. This can be interpreted from the perspective of a sub-regional effect.

A protein is featured by finely structured domains. Variants of different locations are thus potentially associated with various damaging effects and subsequently lead to phenotypical variation. This phenomenon, also known as sub-regional effect, is common mechanism of genetic disorders[23]. LONP1 contains three crucial domains[11,12]. The N domain is involved in substrate recognition. Within this domain, NTD^{5H} plays an important role in substrate unfolding[24]. The A domain includes an NTD^{3H} domain that plays a critical role in the maintenance of optimal ATPase turnover. The A domain also contains a central AAA^{+} module that is involved in ATP binding (α/β domain) and hydrolysis activity (α domain)[24]. The C-terminal serine P domain is involved in substrate proteolysis. Preceding the N domain, there is a 113-amino acid MTS that targets Lon protease to the mitochondria. CODAS syndrome-associated variants were densely located in the AAA+ module, especially the α domain. Four of the five mitochondrial disease-associated missense variants were located at the AAA+ domain and the NTD^{5H} and NTD^{3H} subdomains. In contrast, each of the biallelic variants associated with pure epilepsy had one variant located in the linker domain, and the other variant located in the mitochondrial targeting sequence or P domain, suggesting a sub-regional effect of *LONP1* variants.

It was noted that three patients identified in the present study showed antecedent febrile seizures. Lon protease is responsible for the degradation of glutaminase C, an enzyme that is involved in catalysing the hydrolysis of glutamine to glutamate and ammonia[25]. Dysfunction of Lon protease may decrease the degradation of glutaminase C, thus increasing the concentration of glutamate in neurons. Glutamate is the major excitatory neurotransmitter that has been implicated in the generation of febrile seizures[26,27], which potentially explains the occurrence of febrile seizures in our patients. It is not known whether the previous reported five cases of epilepsy with *LONP1* variants had a history of febrile seizures. We should pay attention to this association in future studies.

This study has several limitations. First, the whole spectrum of *LONP1* variant phenotypes warrants further investigation using larger cohorts. Second, the functional consequences of the variants were not investigated.

In conclusion, *LONP1* is potentially a candidate gene for epilepsy without neurodevelopmental disorders, thereby expanding the phenotypic spectrum of *LONP1*. The sub-regional effects of *LONP1* help to understand the underlying mechanisms of the phenotypic variation.

Materials and methods

Subjects

A total of 450 patients with unexplained epilepsy were recruited. These patients were obtained from the Second Affiliated Hospital of Guangzhou Medical University and Shenzhen Children's Hospital through the China Epilepsy Gene 1.0 Project platform. Clinical data of cases were collected by at least one of the authors. Detailed clinical information, including sex, seizure onset age, types and frequencies of seizures, findings from general and neurological examination, family history, and response to anti-seizure medications, was collected. Brain magnetic resonance imaging (MRI) scans were performed to identify brain structure abnormalities. Video electroencephalography (EEG), which included sleeping recording, hyperventilation, intermittent photic stimulation, and open-close eye tests, was conducted, and the records were reviewed by at least two qualified investigators. Epileptic seizures and epilepsies were diagnosed according to the criteria of the Commission on Classification and Terminology of the ILAE (1981, 2001, 2010, and 2017)[28–34].

Patients with epilepsy with acquired causes, such as immunology, infection, structure, or even known epilepsy genes, were excluded. All enrolled patients were followed up at epilepsy centers for at least 1 year.

This study adhered to the principles of the International Committee of Medical Journal Editors with regard to patient consent for research or participation and was approved by the Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University. Written informed consent was provided by all participants.

Trio-based WES

Blood samples were collected from the probands, their parents and available family members to determine the source of the identified genetic variants. Genomic DNA was extracted from peripheral blood using a QuickGene DNA whole blood kit (Fujifilm). Trio-based WES was conducted on the Illumina HiSeq 2500/4000 platform by BGI-Shenzhen as previously reported[35,36]. Sequencing data were generated by massive parallel sequencing with >125 times average depth and $>98\%$ coverage in the capture region of the chip in order to obtain high-quality reads. The sequencing data were mapped to the Genome Reference Consortium Human Genome build 37 (GRCh37) by Burrows–Wheeler alignment. Single-nucleotide point variants and indels were called with the Genome Analysis Toolkit.

Genetic analysis

To identify candidate causative variants in each trio, we performed a case-by-case analytical approach. For attention to recessive mutations of epilepsy-associated genes, we first prioritized the rare variants with a minor allele frequency (MAF) <0.01 in the 1000 Genomes Projects, Exome Aggregation Consortium, and gnomAD[37]. Potentially pathogenic variants were defined as frameshift, nonsense, canonical splice site, initiation codon, and missense mutations predicted to be damaging by in silico tools (http://www.genemed.tech/varcards/). Then, we screened potential disease-causing variants in each case using five models: (1) epilepsy-associated gene variants, (2) de novo dominant variants, (3) autosomal recessive inheritance model, (4) X-linked model, and (5) co-segregated variants. Possible novel epilepsy genes were considered if a gene presented recurrent de novo variants, biallelic variants, hemizygous variants, and variants with segregations. Sanger sequencing was performed to confirm potential clinically significant variants. All *LONP1* variants were annotated based on the transcript NM_004793.4.

Mutation analyses

To evaluate the damaging effects of candidate variants, the structure of Lon protease was modelled by using AlphaFold (https://alphafold.ebi.ac.uk/). PyMOL Molecular Graphics System 2.3.2 software was used for three-dimensional protein structure visualization and analysis. The I-Mutant 3.0 server was used to predict protein stability changes. The changes in protein stability were assessed using the free energy stability change value ($\Delta\Delta G$, Kcal/mol).

The MAFs of the epilepsy-associated and CODAS syndrome and mitochondrial disease-associated variants were compared with those of the "benign/likely benign" variants from the NCBI-ClinVar database (www.ncbi.nlm.nih.gov/clinvar/). MAFs were obtained from the GnomAD database.

Literature review

To evaluate the genotype-phenotype correlation, we systematically reviewed *LONP1* variations in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) and the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php; Professional 2023.1 version).

Statistical analysis

R statistical software (version 4.3.2) was used to process the data. The Mann-Whitney test was used to compare the frequencies between the benign missense variants and those of the epilepsy-associated variants, CODAS syndrome and mitochondrial disease-associated variants. A p-value <0.05 was considered statistically significant.

Data availability

The data are not publicly available due to privacy or ethical restrictions. For further information, please contact the corresponding author.

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References

1. Wang, N., Gottesman, S., Willingham, M. C., Gottesman, M. M. & Maurizi, M. R. A human mitochondrial ATP-dependent protease that is highly homologous to bacterial lon protease. *Proc. Natl. Acad. Sci.* **90**, 11247–11251 (1993).
2. Ondrovičová, G. et al. Cleavage site selection within a folded substrate by the ATP-dependent lon protease. *J. Biol. Chem.* **280**, 25103–25110 (2005).
3. Gilkerson, R. et al. The mitochondrial nucleoid: integrating mitochondrial DNA into Cellular Homeostasis. *Cold Spring Harb Perspect. Biol.* **5**, a011080–a011080 (2013).
4. Shin, C. S. et al. LONP1 and mtHSP70 cooperate to promote mitochondrial protein folding. *Nat. Commun.* **12**, 265 (2021).
5. Quirós, P. M. et al. ATP-dependent Lon protease controls tumor bioenergetics by reprogramming mitochondrial activity. *Cell. Rep.* **8**, 542–556 (2014).
6. Shebib, S. M. et al. Newly recognized syndrome of cerebral, ocular, dental, auricular, skeletal anomalies: CODAS syndrome—a case report. *Am. J. Med. Genet.* **40**, 88–93 (1991).
7. Strauss, K. A. et al. CODAS Syndrome is Associated with mutations of LONP1, encoding mitochondrial AAA + lon protease. *Am. J. Hum. Genet.* **96**, 121–135 (2015).
8. Besse, A., Brezavar, D., Hanson, J., Larson, A. & Bonnen, P. E. LONP1 de novo dominant mutation causes mitochondrial encephalopathy with loss of LONP1 chaperone activity and excessive LONP1 proteolytic activity. *Mitochondrion* **51**, 68–78 (2020).
9. Nimmo, G. A. M. et al. Bi-allelic mutations of LONP1 encoding the mitochondrial LonP1 protease cause pyruvate dehydrogenase deficiency and profound neurodegeneration with progressive cerebellar atrophy. *Hum. Mol. Genet.* **28**, 290–306 (2019).
10. Wang, J. et al. Epilepsy-associated genes. *Seizure* **44**, 11–20 (2017).
11. Wlodawer, A., Sekula, B., Gustchina, A. & Rotanova, T. V. Structure and the mode of activity of Lon proteases from diverse organisms. *J. Mol. Biol.* **434**, 167504 (2022).
12. Shin, M. et al. Structures of the human LONP1 protease reveal regulatory steps involved in protease activation. *Nat. Commun.* **12**, 3239 (2021).
13. Inui, T. et al. A novel mutation in the proteolytic domain of LONP1 causes atypical CODAS syndrome. *J. Hum. Genet.* **62**, 653–655 (2017).
14. Al-Dewik, N. et al. Clinical exome sequencing in 509 Middle Eastern families with suspected mendelian diseases: the Qatari experience. *Am. J. Med. Genet. A* **179**, 927–935 (2019).
15. Dikoglu, E. et al. Mutations in *LONP1*, a mitochondrial matrix protease, cause CODAS syndrome. *Am. J. Med. Genet. A* **167**, 1501–1509 (2015).
16. Stranneheim, H. et al. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. *Genome Med.* **13**, 40 (2021).
17. Tang, Y. et al. The first case report of CODAS syndrome in Chinese population caused by two LONP1 pathogenic mutations. *Front. Genet.* **13**, 1031856 (2023).
18. Khan, A. O. & AlBakri, A. Clinical features of LONP1 -related infantile cataract. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* **22**, 229–231 (2018).
19. Peter, B. et al. Defective mitochondrial protease LonP1 can cause classical mitochondrial disease. *Hum. Mol. Genet.* **27**, 1743–1753 (2018).
20. Hannah-Shmouni, F., MacNeil, L., Brady, L., Nilsson, M. I. & Tarnopolsky, M. Expanding the clinical spectrum of LONP1-Related mitochondrial cytopathy. *Front. Neurol.* **10**, 981 (2019).
21. Kim, J. E., Park, H., Kim, T. H. & Kang, T. C. LONP1 regulates mitochondrial accumulations of HMGB1 and Caspase-3 in CA1 and PV neurons following Status Epilepticus. *Int. J. Mol. Sci.* **22**, 2275 (2021).
22. Venkatesh, S. et al. Mitochondrial LonP1 protects cardiomyocytes from ischemia/reperfusion injury in vivo. *J. Mol. Cell. Cardiol.* **128**, 38–50 (2019).
23. Liao, W. P. et al. Sub-molecular mechanism of genetic epilepsy. *Front. Mol. Neurosci.* **15**, 958747 (2022).
24. Cheng, I. et al. Identification of a region in the N-Terminus of Escherichia coli Lon that affects ATPase, substrate translocation and proteolytic activity. *J. Mol. Biol.* **418**, 208–225 (2012).
25. Kita, K., Suzuki, T. & Ochi, T. Diphenylarsinic Acid promotes degradation of glutaminase C by mitochondrial lon protease. *J. Biol. Chem.* **287**, 18163–18172 (2012).
26. Sarlo, G. L. & Holton, K. F. Brain concentrations of glutamate and GABA in human epilepsy: a review. *Seizure* **91**, 213–227 (2021).
27. Crespo, M., León-Navarro, D. A. & Martín, M. Glutamatergic system is affected in brain from an Hyperthermia-Induced seizures rat model. *Cell. Mol. Neurobiol.* **42**, 1501–1512 (2022).
28. Engel, J. ILAE classification of epilepsy syndromes. *Epilepsy Res.* **70**, 5–10 (2006).
29. Scheffer, I. E. et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and terminology. *Epilepsia* **58**, 512–521 (2017).
30. Riney, K. et al. International League Against Epilepsy classification and definition of epilepsy syndromes with onset at a variable age: position statement by the ILAE Task Force on Nosology and definitions. *Epilepsia* **63**, 1443–1474 (2022).
31. Fisher, R. S. et al. Operational classification of seizure types by the International League against Epilepsy: position paper of the ILAE Commission for classification and terminology. *Epilepsia* **58**, 522–530 (2017).
32. Berg, A. T. et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on classification and terminology, 2005–2009. *Epilepsia* **51**, 676–685 (2010).
33. Pressler, R. M. et al. The ILAE classification of seizures and the epilepsies: modification for seizures in the neonate. Position paper by the ILAE Task Force on neonatal seizures. *Epilepsia* **62**, 615–628 (2021).
34. Proposal for Revised Classification of Epilepsies and Epileptic Syndromes. Commission on classification and terminology of the International League against Epilepsy. *Epilepsia* **30**, 389–399 (1989).
35. Wang, J. et al. *UNC13B* variants associated with partial epilepsy with favourable outcome. *Brain* **144**, 3050–3060 (2021).
36. Shi, Y. W. et al. Synaptic clustering differences due to different GABRB3 mutations cause variable epilepsy syndromes. *Brain* **142**, 3028–3044 (2019).
37. Qiao, J. D. et al. *UNC13B* and focal epilepsy. *Brain* **145**, e13–e16 (2022). Reply.

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Author contributions

LB and SXW conceived and designed the study, analyzed the data and wrote the paper. LSX and HN collected and analyzed the data, performed data analysis, and wrote the paper. LXG, LJX, and HWG collected and performed statistical analysis. LXR and LWP analyzed and interpreted the data.

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Competing interests
The authors declare no competing interests.

Ethics statement
This study was approved by the ethics committee of the Second Affiliated Hospital of Guangzhou Medical University (approval ethics number 2020-hs-49).

Consent to participate
Informed consent was obtained from all individual participants or their parents included in the study.

Additional information
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