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CLPP Gene Variants Causing Perrault Syndrome Type 3 in Han Chinese Families: A Genotype-Phenotype Study

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Abstract

Background Perrault syndrome is a rare autosomal recessive disorder characterized by sensorineural hearing loss (SNHL) and primary ovarian insufficiency (POI) secondary to ovarian dysgenesis. However, the mutation spectrum of disease-causing genes for Perrault syndrome in the Chinese population remains poorly understood. In this study, we report on two Chinese families with Perrault syndrome type 3 caused by novel *CLPP* gene variants. We also conducted a comprehensive literature review of *CLPP* gene variants in Perrault syndrome type 3 to elucidate genotype-phenotype associations.

Methods Using Whole Genome Sequencing (WGS) data, two pedigrees with Perrault syndrome type 3 were ascertained in the Chinese Deafness Genetics Cohort through genotype-driven analysis. Variants were validated using Sanger sequencing and copy number quantification methods. In vitro analysis of splice site variants in the *CLPP* gene using the minigene assay.

Results Two Han Chinese families were ascertained: one with compound heterozygous variants (c.270 + 1G > C and c.355A > C [p. Ile119Leu]) and the other with missense variant (c.400G > C [p. Asp134His]) together with a large deletion in *CLPP*. In vitro minigene assays confirmed that the c.270 + 1G > C variant causes intron 2 retention and an alternative 5′ splice site in exon 2, leading to protein alteration. Among 33 Perrault syndrome type 3 patients in literature, 97% (31/32) had hearing loss, 55% (16/29) neurological disease, and 71% (15/21) females had POI. Including our 4 novel variants, 21 pathogenic *CLPP* gene variants have been reported, with 57% (12/21) missense and 43% (9/21) truncating variants, mainly in the ATP-dependent Clp protease proteolytic subunit. Biallelic truncating or missense plus truncating genotypes showed higher rates of neurological disease ($p = 0.001$), but no significant difference in hearing loss incidence compared to biallelic missense genotypes was observed.

Conclusion This study highlights the challenges in diagnosing Perrault syndrome due to its genetically and clinically heterogeneity. By exploring novel variants and establishing genotype-phenotype correlations, we aim to improve the genetic diagnosis and consultation for this complex disorder.

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Keywords *CLPP*, Perrault syndrome type 3, Genotype-phenotype correlation, Genotype-driven

Introduction

Perrault syndrome is a rare autosomal recessive disorder characterized by sensorineural hearing loss (SNHL) and primary ovarian insufficiency (POI) due to ovarian dysgenesis in females. Since first described by Perrault et al. in 1951 [1], the syndrome has been reported in various populations worldwide, with an estimated prevalence of 1 in 1,000,000 individuals (https://www.orpha.net). The genetic basis of Perrault syndrome is complex and remains poorly understood. To date, variants in eight genes have been identified as the underlying cause of the syndrome, including *CLPP*, *ERAL1*, *GGPS1*, *HARS2*, *HSD17B4*, *LARS2*, *RMND1*, and *TWNK* [2]. Among these genes, *CLPP* biallelic variant associated with Perrault syndrome type 3 (OMIM 614129) was first described as a non-syndromic hearing loss mapped to a 4.17 Mb region on chromosome 19p13, and was identified as the causative gene of Perrault syndrome type 3 in 2013 [3–5]. The *CLPP* gene encodes a protein (45 kDa) with 277 residues, which is called caseinolytic mitochondrial matrix peptidase proteolytic subunit, and is involved in the proteolysis of damaged and misfolded proteins, ribosome-stalled proteins, as well as of regulatory proteins [6–7]. CLPP, which belongs to the peptidase family S14, is a nuclear-encoded mitochondrial protease that is targeted to the mitochondrial matrix via an N-terminal targeting sequence [8]. Based on data from the Genotype-Tissue Expression (GTEx) database (https://www.genome.gov/Funded-Programs-Projects/Genotype-Tissue-Expression-Project), the *CLPP* gene exhib its notable expression across multiple tissues [6]. CLPP shows high expression in tissues and organs with high energy demands, such as the heart, muscles, and liver. Additionally, CLPP expression has been detected in other tissues, including the brain, cochlea, ovary, kidney, pancreas, and testis. The CLPP substrate pool is extensive, comprising proteins involved in essential mitochondrial processes, such as the Krebs cycle, oxidative phosphorylation, translation, fatty acid metabolism, and amino acid metabolism [9]. Consistent with its important role in Perrault syndrome, *Clpp* knockout mice also exhibited hearing loss, growth retardation and reproductive abnormalities, including ovarian dysfunction and male infertility [5, 10–12].

The genetic and clinical heterogeneity of Perrault syndrome makes its diagnosis particularly challenging [2, 5]. Advances in genetic testing technologies, such as next-generation sequencing (NGS), have significantly improved the ability to identify genetic variants associated with this syndrome [13, 14]. NGS allows for the comprehensive analysis of multiple genes simultaneously, which is crucial given the diverse genetic causes of Perrault syndrome. For instance, variants in genes such as *CLPP*, *ERAL1*, *GGPS1*, *HARS2*, *HSD17B4*, *LARS2*, *RMND1*, and *TWNK* have been associated with the disorder, but these account for only about half of the diagnosed cases, indicating that additional genetic factors are likely involved [2]. Moreover, the clinical manifestations of Perrault syndrome are highly variable, encompassing a range of phenotypes, including SNHL, POI, and neurological features such as learning difficulties, developmental delay, cerebellar ataxia, and motor or sensory peripheral neuropathy [5, 15–16]. The clinical diagnosis of Perrault syndrome is particularly challenging because male patients typically present solely with SNHL, whereas female patients often do not manifest symptoms of POI until after puberty. This delay in specific symptom presentation makes it difficult to achieve an accurate diagnosis in the early stages. Consequently, molecular diagnosis becomes essential for the early identification of Perrault syndrome. In this study, we aim to identify novel *CLPP* variants in Chinese families affected by Perrault syndrome and compare these findings with existing literature data.

Materials and methods

Pedigrees and participators

We recruited two patients from separate families in the Chinese Deafness Genetics Cohort. A detailed medical history was collected, encompassing information on age of onset, relevant clinical manifestations, clinical tests, severity of hearing loss, history of cochlear implant surgery, rehabilitative outcomes, noise exposure, ototoxic drug exposure, and family history. Ethical approval for this study was obtained from the Institutional Review Board of West China Hospital, Sichuan University (2021-190), and informed consent was obtained from all participants or their legal guardians.

WGS and data analysis

Peripheral blood DNA was extracted from both patients and family members, and the patient with hearing loss underwent WGS. The sequencing was performed using DNBSEQ-T7 sequencing instruments (MGI, Shenzhen, China), generating paired-end reads of 150 bp in length. The Burrows-Wheeler Aligner (BWA, v0.5.10) was used to map the reads to the human reference genome (GRCh38). An in-house variant-calling workflow, based on the GATK best practice, was developed for variant identification. Variants were annotated with information on variant quality, details, gene information, sequence database, allele state, and allele frequency

[17]. For variant analysis, we applied the following filters: (1) Genotype quality ≥ 20, read depth ≥ 6, and alternate allele frequency > 0.1; (2) Popmax minor-allele frequency (MAF) ≤ 0.5% in the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org) and retention of coding variants, splice regions, or selected pathogenic or likely pathogenic variants in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), Human Gene Mutation Database (HGMD) (https://www.hgmd.cf.ac.uk), the Deafness Variation Database (DVD) (https://deafnessvariationdatabase.org), or in-house hearing loss variants database. The pathogenic potential of the identified variants was classified and interpreted according to the ACMG/AMP variant interpretation guidelines for genetic hearing loss [18]. Additionally, we used CNVnator and Manta to detect copy number variants (CNVs) in the WGS data. CNVs were filtered based on the following criteria: (1) Allele frequencies < 1% in gnomAD and the 1000 Genomes Project (https://www.1000genomes.org); (2) ≤ 50% reciprocal overlap with the Database of Genomic Variants (DGV) (http://dgv.tcag.ca); (3) ≤ 25% overlap with repetitive and low-complexity regions. The pathogenicity of CNVs was interpreted in accordance with the ACMG/AMP variant interpretation guidelines [19].

Sanger sequencing and *CLPP* copy number quantification

The variants identified in the proband through WGS were validated in both the affected patients and their unaffected family members using Sanger sequencing. Primer sequences for polymerase chain reaction (PCR) amplification were designed using Primer3 software (http://primer3.ut.ee/), and the primer details can be found in Supplementary Table 1 A. The resulting PCR products were analyzed for quality using agarose gel electrophoresis. Subsequently, the PCR products were purified and subjected to Sanger sequencing using standard protocols.

To validate CNVs, a quantitative PCR (qPCR) method was developed. Primer pairs located across gene exons were designed (Primers: Supplementary Table 1B). Genomic DNA was extracted from the proband and his family members, and qPCR reactions were performed using TaKaRa Taq™ Hot Start DNA polymerase (Code No. R007Q) in a 15μL system. The qPCR analysis was performed using the Ct method (also known as the $2^{-\Delta\Delta Ct}$ method), and the obtained results were compared with a normal reference standard to determine the CNVs. The experiment was repeated three times.

In vitro splicing analysis

A minigene assay was used to assess the impact of the novel *CLPP* splice site variant on mRNA splicing: (1) The recombinant plasmid was constructed by inserting a wild-type *CLPP* gene fragment (chr19:6361792-chr19:6363124), encompassing exons 2 and 3 and intron 2, into the pSPL3 vector via homologous recombination method (Primers: Supplementary Table 1 C; Fig. 2E). (2) Next, site-directed mutagenesis was performed on the wild-type template to generate a mutant plasmid utilizing the NovoRec® plus Kit (Novoprotein, Code NR005) and DpnI digestion (Primers: Supplementary Table 1D). (3) The recombinant plasmid was transformed into *E. coli* DH5α cells (Takara, Code No.9057) for colony selection and verified by Sanger sequencing (Primers: Supplementary Table 1E; Fig. 2E). The plasmid was then amplified. (4) The recombinant plasmid was transfected into COS-7 cells, which were seeded at a density of 5 × 10^4 cells per well in a 6-well plate and allowed to adhere overnight, using a lipofection method (Thermo Fisher Scientific, Code No. L3000015) according to the manufacturer's protocol. (5) Total RNA was extracted from transfected COS-7 cells 48 h post-transfection using an RNA extraction kit (Tiangen, Code No. DP451), and cDNA was synthesized using a Reverse Transcription Kit (Takara, Code No. RR047A). (6) The PCR products were resolved on a 1.5% agarose gel and verified by Sanger sequencing (Primers: Supplementary Table 1 F; Fig. 2E).

Statistics

The data were analyzed using SPSS version 25.0 (IBM SPSS Statistics for Windows). Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD). Fisher's exact test was used to evaluate the association between two categorical variable rates. A significance level of $p < 0.05$ was considered statistically significant.

Results

Clinical findings

In Family 1 (Fig. 1A), the proband (II-1), a 16-year-old male, was found to have bilateral profound hearing loss at age 3. As a result, he underwent cochlear implant surgery at the age of 6. After experiencing feeding difficulties immediately following birth, the child began walking at over 2 years old but continues to exhibit persistent unsteadiness and an increased risk of falls, especially while running. By the age of 5, he was diagnosed with intellectual disability. In Family 2 (Fig. 1B), the proband (II-1), an 11-year-old female, was found to have bilateral profound hearing loss at age 2. No abnormalities were found in other systems. None of the other family members displayed any symptoms. A summary of all clinical details can be found in Table 1.

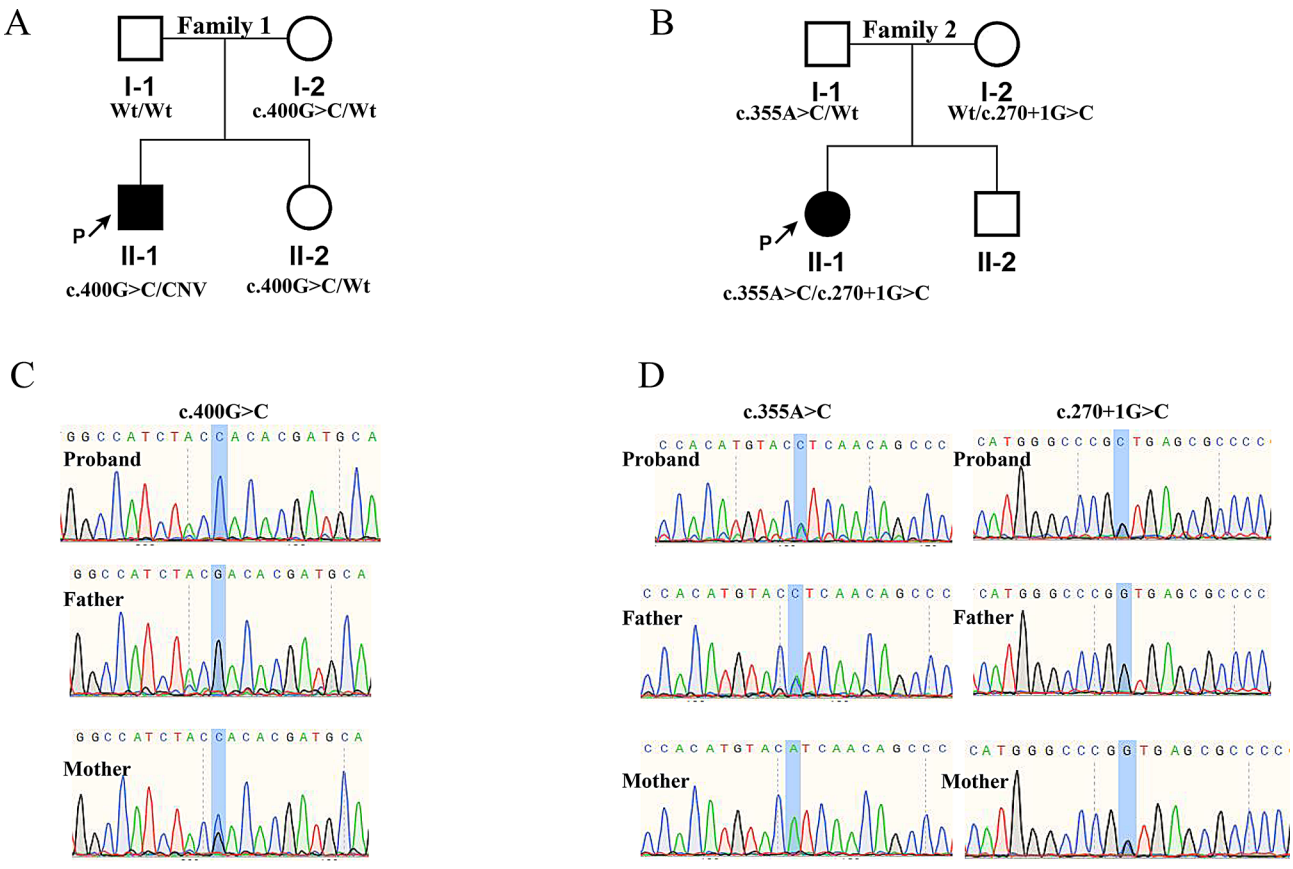

Fig. 1 The pedigree, Sanger sequencing results of Family 1 and Family 2 are presented. **A**)The pedigree of Family 1; **B**) The pedigree of Family 2; **C**) Sanger sequencing results of Family 1; **D**) Sanger sequencing results of Family 2

Table 1 Clinical features for probands

Patient ID	Family 1:II-1	Family 2:II-1
Sex	Male	Female
Age(years old)	16	11
Karyotype	46,XY	NA
Hearing loss	Y	Y
Hearing loss_onset age(years old)	3	2
Hearing loss_severity	Profound	Profound
Primary ovarian insufficiency	/	NA
Mental retardation	Y	Normal
Learning difficulty	Y	Normal
Developmental delay	Y	Normal
Ataxia, cerebellar	Y	Normal
Epilepsy	N	Normal
Short stature	Y	Normal
Microcephaly	Normal	Normal
Peripheral neuropathy	Normal	Normal
Motor neuron dysfunction	Normal	Normal
Others	Scoliosis	NA

Note Y, yes; N, no; NA, not available

Genetic findings

In Family 1, a novel missense variant NM_006012.4: c.400G > C (NP_006003.1: p. Asp134His) was detected in the proband (II-1), in combination with a large deletion of exons 1 to 6 (chr19:6059838–6992969) in *CLPP* gene. Details are provided in Figs. 1A and 2A. The missense variant was inherited from his unaffected mother (Fig. 1C). This variant was not detected in our normal hearing controls, which consist of 7,254 unrelated adults over the age of 18 years without self-reported hearing impairment, nor in the gnomAD database. This variant is conserved across multiple species, and protein structure predictions suggest that it may have deleterious effects (Fig. 2C and D). According to the ACMG/AMP guidelines [18], the c.400G > C variant was interpreted as a likely pathogenic variant, supported by evidence from PM3, PM2, PP4 and PP3. The large heterozygous deletion is a CNV was not detected in his unaffected family members (Fig. 2B). This CNV was also not detected in our normal hearing controls and the gnomAD database. Based on the ClinGen CNV Pathogenicity Calculator, the CNV is classified as a pathogenic variant (total score: 2.05 points).

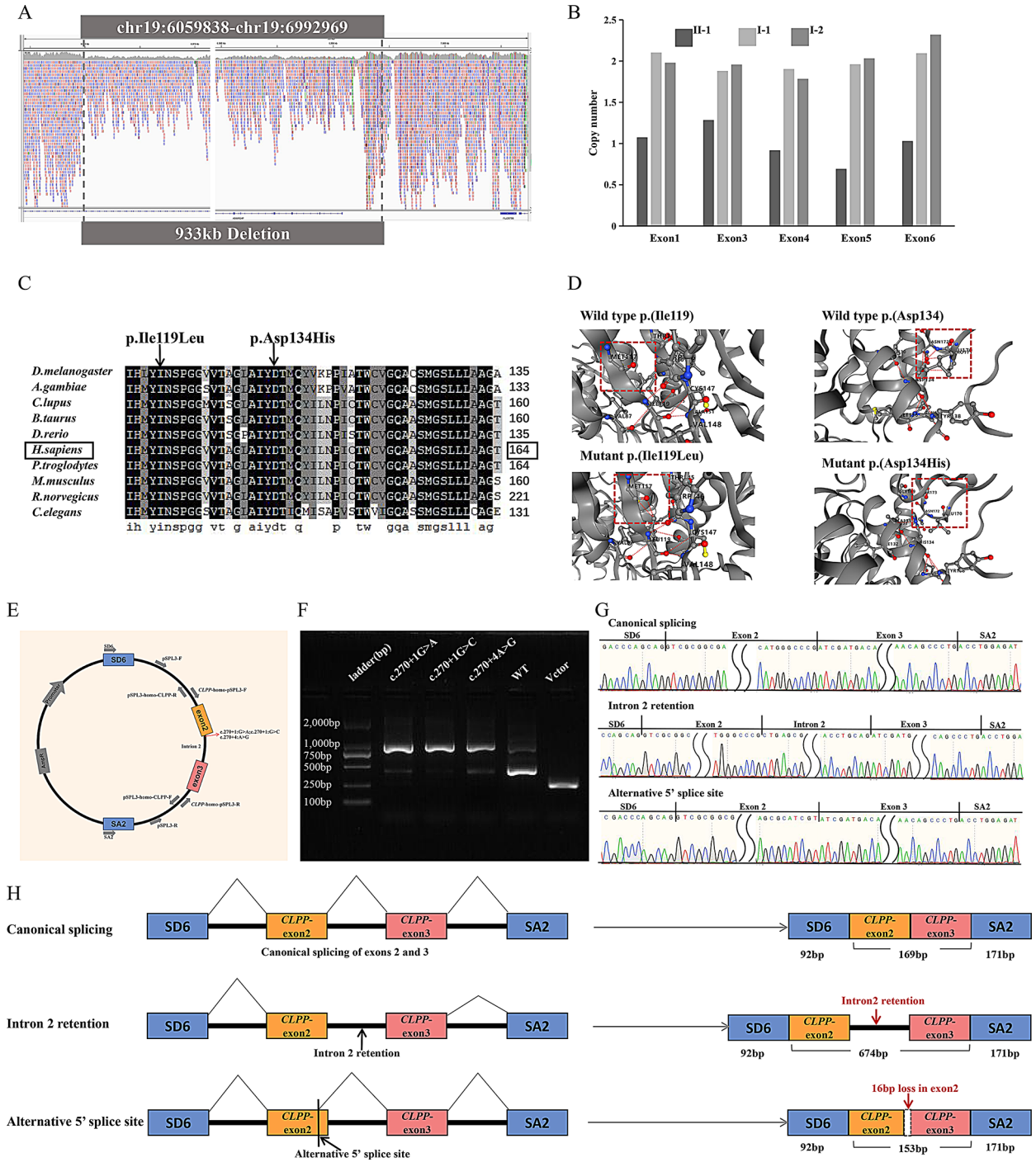

Fig. 2 Validation of copy number variant (CNV), conservation and functional prediction of missense variants, and in vitro analysis of splice site variant. **A**) BAM of the CNV in the *CLPP* gene found in proband of Family 1; **B**) Copy number quantification result in Family 1; **C**) The conservative analysis in multiple species for p.Ile119Leu and p.Asp134His; **D**) Protein structure predictions for p.Ile119Leu and p.Asp134His; **E**) Schematic presentation of the exon-splicing-assay vector used in COS-7 cells for the evaluation of the predicted splice site variant; **F**) Splicing-assay products were separated by agarose gel; **G**) Sanger sequencing after the product were recovered; **H**) the schematic diagram of splicing patterns

In Family 2, compound heterozygous variants in the *CLPP* gene were identified. The proband (II-1) carried the variants NM_006012.4: c.270 + 1G > C and NM_006012.4: c.355 A > C (NP_006003.1: p. Ile119Leu). The proband's mother was a carrier of the c.270 + 1G > C variant, while the father was a carrier of the c.355 A > C variant (Fig. 1B and D). Both variants in Family 2 were not observed in our normal hearing controls and the gnomAD database.

This missense variant c.355 A > C is conserved across multiple species, and protein structure predictions suggest that it may have deleterious effects (Fig. 2C and D). According to the ACMG/AMP guidelines [18], the c.270 + 1G > C variant was classified as pathogenic, supported by evidence from PVS1, PS1_Supporting, PM2, and PP4. Similarly, the c.355 A > C variant was interpreted as likely pathogenic, supported by PM3, PM2, PP4, and PP3 evidence.

Minigene assay results

Our RT-PCR analysis of minigene splicing patterns revealed distinct differences between the wild-type and mutant-type samples. The splicing-assay products of the wild-type, c.270 + 1G > A, c.270 + 1G > C, c.270 + 4 A > G, and vector were resolved by size on a 1.5% agarose gel (Fig. 2F). Further validation was performed using Sanger sequencing (Fig. 2G). The splicing patterns are shown in Fig. 2H. Consequently, in the wild-type sample, two distinct splicing patterns were observed (Fig. 2F, G and H). The smaller band, with a size of 432 base pairs, corresponds to the canonical splicing of exons 2 and 3. The larger band, which includes retained intron 2, has a size of 937 base pairs. This finding aligns with the study by Jenkinson et al. [5], which reported that, for the wild-type sequence, 62% (23/37) of cloned transcripts exhibited canonical splicing of exons 2 and 3, while 30% (11/37) of clones retained intron 2, and 8% (3/37) contained other aberrant transcripts. The canonical splicing of exons 2 and 3 was identified as the primary splicing pattern in the wild-type sample in both studies.

The mutant-type samples (c.270 + 1G > A, c.270 + 1G > C, and c.270 + 4A > G) also exhibited two splicing patterns (Fig. 2F, G and H). The smaller band was characterized by the usage of an alternative 5' splice site, resulting in a 16-bp deletion in exon 2 and a final band size of 416 base pairs. The larger band was characterized by the retention of intron 2, with a size of 937 base pairs. The observed alternative 5' splice site usage aligns with the predictions made by SpliceAI (Supplementary Fig. 1). Intron 2 retention was identified as the primary splicing pattern in this study. Similarly, in the study by Jenkinson et al. [5], intron 2 retention was also the predominant splicing pattern for the c.270 + 1G > A and c.270 + 4 A > G variants.

Cochlear implant outcomes

In this study, patient II-1 from Family 1 underwent left-sided cochlear implantation (CI) at the age of 6. Following the surgical intervention and subsequent speech rehabilitation, the patient is now able to distinguish and recognize a wide range of voices in daily life, enabling him to understand instructions effectively. Furthermore, he has developed the ability to communicate simply in daily interactions and can articulate several words, although his speech clarity remains somewhat impaired.

Clinical characteristics of Perrault syndrome type 3 patients reported in the literature

The literature review revealed 33 patients with Perrault syndrome type 3 from 15 families. Among these 33 patients, approximately 97% (31/32) presented with hearing loss, 55% (16/29) had neurological diseases, and 71% (15/21) of the female patients experienced POI. Further details are provided in Supplementary Table 2. In terms of hearing loss, the age of onset ranged from congenital to childhood, with 79% (23/29) experiencing pre-lingual onset and 21% (6/29) post-lingual onset. The severity of hearing loss varied from moderate to profound. Among the 15 patients with POI, 40% (6/15) exhibited primary amenorrhea, while 60% (9/15) displayed secondary amenorrhea. The age range for secondary amenorrhea was between 16 and 33 years old, with a mean age of 22 ± 4.75 years ($n = 8$). Pelvic ultrasound findings were available for 11 female patients with POI, revealing a spectrum of ovarian and uterine abnormalities. Notably, 18% (2/11) had normal ovaries, 18% (2/11) had the absence of one or both ovaries, and 18% (2/11) presented with streak ovaries. Additionally, 36% (4/11) had small ovaries, and 9% (1/11) showed the absence of follicles. Regarding the uterus, 45% (5/11) were normal, 27% (3/11) had small uteruses, and 27% (3/11) had rudimentary uteruses. Among the 16 patients with neurological disease, a range of conditions was observed. The most prevalent condition was ataxia, affecting 69% (11/16) of patients, followed by spasticity and movement disorder, each present in 63% (10/16) of patients. Epilepsy was observed in 50% (8/16) of patients, while intellectual disability and developmental delay were both present in 44% (7/16) of cases. Other symptoms, such as learning difficulties, dysarthria, behavioral problems, and dysphagia, were found in 31% (5/16) of cases. Autism and sensory neuropathy less prevalent, each affecting 13% (2/16) of patients. Attention deficit disorder was the least common, affecting only 6% (1/16) of patients. Brain magnetic resonance imaging (MRI) findings were available for 12 patients with neurological disease, revealing that 8% (1/12) had normal results, while 92% (11/12) exhibited signs of leukoencephalopathy. Height and head circumference evaluation in 17 individuals showed that 47% (8/17) had short stature and 29% (5/17) had microcephaly. Additionally, other symptoms such as dysmorphic features were observed in 4 individuals, while strabismus, inguinal hernia, diabetes mellitus, hypothyroidism, and azoospermia were each observed in 1 patient. Further details are provided in Supplementary Table 3.

Correlation between *CLPP* genotype and phenotype in Perrault syndrome type 3 patients

Among a total of 35 Perrault syndrome type 3 patients (including 2 patients in this study), we compiled a total of 18 *CLPP* pathogenic single nucleotide variants (SNVs) and 3 CNVs in the *CLPP* gene. These included 57% (12/21) missense variants, 14% (3/21) nonsense or frameshift variants, 19% (4/21) CNVs, and 10% (2/21) splicing variants. Missense variants accounted for 57% (12/21), while truncating variants accounted for 43% (9/21). Further details are provided in Supplementary Table 4. The pathogenic variants were distributed throughout the gene, with a concentration in the ATP-dependent Clp protease proteolytic subunit. Details are shown in Fig. 3A. When comparing biallelic truncating or missense plus truncating genotype with biallelic missense genotypes, a higher incidence rate of neurological disease was observed ($p = 0.001$), while no statistically significant difference was observed in the incidence rates of hearing loss. Details are provided in Table 2 and Supplementary Table 5. Additionally, our research has revealed significant phenotypic heterogeneity, with an association illustrated in Fig. 3B.

Discussion

In this study, we report on two patients with Perrault syndrome type 3 from distinct Chinese families harboring novel *CLPP* SNVs and CNVs. Additionally, we conducted an in-depth literature review to clarify the correlation between *CLPP* gene variants and the clinical features of Perrault syndrome. According to the literature, a total of 33 patients from 15 families with Perrault syndrome type 3 have been reported. The phenotype of Perrault syndrome type 3 exhibits considerable heterogeneity in onset time and clinical presentation. Hearing loss typically manifests pre-lingually and varies in severity from moderate to profound. Many female patients also present with POI, encompassing primary and secondary amenorrhea, along with diverse ovarian and uterine abnormalities detected by pelvic ultrasound. Neurological symptoms are broad and variable. Importantly, we identified additional coexisting phenotypes in Perrault syndrome type 3 patients. Patient II-1 from Family 1 presented with scoliosis (Supplementary Fig. 2), a phenotype not previously reported in association with the *CLPP* gene in the existing literature. We considered three potential explanations: First, scoliosis could represent a novel phenotype associated with the *CLPP* gene. Second, it could be caused by other pathogenic genes. However, WGS analysis did not reveal any candidate variants associated with scoliosis. Third, we also considered acquired factors such as sitting posture and daily habits that might contribute to scoliosis development. Based on earlier findings, dysmorphic features were observed in 4 individuals [15, 20]. Additionally, strabismus [15], inguinal hernia [15], diabetes mellitus [15], hypothyroidism [15], and azoospermia [15] were each observed in 1 patient. Although azoospermia was observed in only 1 patient among these additional phenotypes, it is noteworthy because it has also been observed in animal studies [12].

In our review of the previous literature, we reviewed a total of 18 pathogenic SNVs and 3 CNVs in the *CLPP* gene. These variants predominantly cluster within the ATP-dependent Clp protease proteolytic subunit domain, which is essential for CLPP protein function. This domain serves two primary roles. First, it interacts with the ATP-dependent unfoldase CLPX, forming a complex that facilitates CLPX-mediated proteolytic activity [21–25]. Second, it exhibits peptidase activity through its catalytic triad [26]. The presence of these variants in these critical regions suggests potential disruptions to CLPP protein function and underscores their importance in elucidating the molecular mechanisms underlying Perrault syndrome type 3.

To further investigate the genotype-phenotype correlation of this syndrome, we separated patients into two groups based on the type of the variants. Biallelic missense genotypes were considered as proxies for genotypes that would cause mild loss of function, while biallelic truncating or missense plus truncating genotypes were considered as proxies for genotypes that may cause severe loss of function. As a result, our findings revealed a notable association between biallelic truncating or missense plus truncating *CLPP* variants and a higher incidence rate of the polyneuropathy phenotype ($p < 0.05$). This result aligns with previous studies that have demonstrated the deleterious effects of truncating variants on protein function, leading to severe clinical manifestations [27]. Additionally, we did not observe a significant difference in the incidence rates of the hearing loss phenotype between the two genotype groups. This suggests that even minor changes in protein function can result in the occurrence of the phenotype.

Currently, there is no effective drug treatment for Perrault syndrome, with symptomatic supportive care being the mainstay of management. CI is a leading treatment for severe or profound hearing loss, provided that the auditory nerves and spiral ganglion are intact. CI is particularly effective in congenital cases or post-lingual adults [28]. Success with CI has been documented in a patient with *CLPP*-related auditory neuropathy spectrum disorder (ANSD), in whom CI improved vocalization after psychomotor and speech rehabilitation [29]. In our study, a male patient (II-1, Family 1) underwent CI at the age of 6 for congenital profound hearing loss. He had a good auditory response, but his speech rehabilitation was limited due to intellectual disability. ANSD in

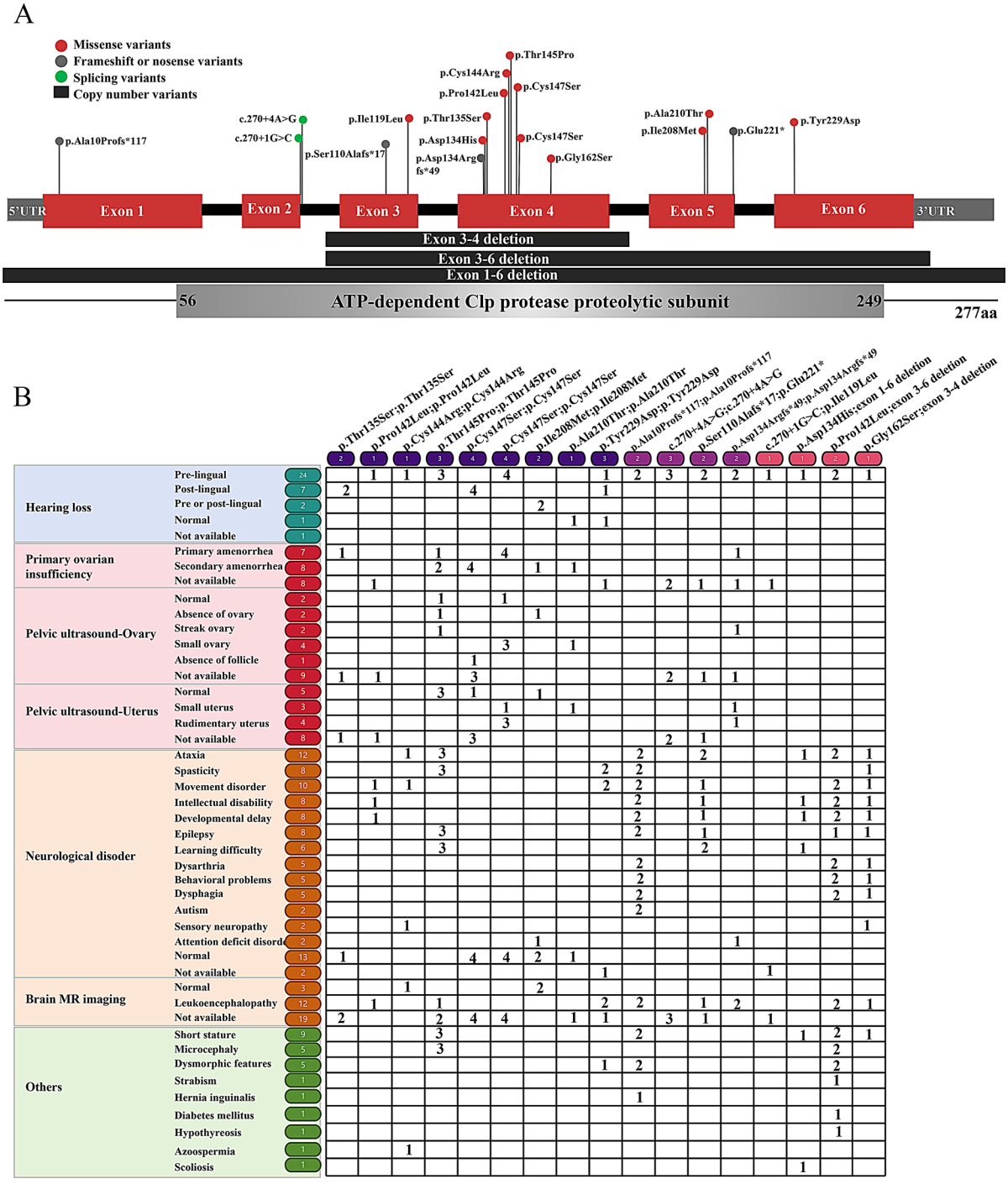

Fig. 3 The mutation spectrum of *CLPP*, genotype-phenotype correlation spectrum of *CLPP* and Perrault syndrome type 3. **A**) Gene structure of *CLPP* and protein domain of CLPP; **B**) genotype-phenotype correlation spectrum of *CLPP* and Perrault syndrome type 3

Perrault syndrome includes 1 case related to *CLPP* variants, as well as other cases linked to *TWNK* variants (Perrault syndrome type 5) and *HARS2* variants (Perrault syndrome type 2) [30–31]. Careful evaluation of intellectual disability is crucial before considering CI in patients with Perrault syndrome.

Current mechanistic studies of Perrault syndrome type 3 primarily focus on hearing loss and reproductive system, with limited attention given to the nervous system. Jenkinson et al. observed CLPP expression in the organ of Corti supporting and hair cells in P3 mice [5]. Gispert et al. found that *Clpp* gene knockout (KO) in mice aged 12-18 months resulted in hearing loss, growth

Table 2 Genotype and phenotype correlation

Clinical Feature	All Genotype	Group 1	Group 2	Group 1 vs. Group 2
	%(*n*/*N*)*	%(*n*/*N*)	%(*n*/*N*)	*p* (Fisher's exact test)
Hearing loss	**100%(33/33)**	**95%(18/19)**	**100%(14/14)**	**1**
Neurological disease	**57%(17/30)**	**35%(7/20)**	**100%(10/10)**	**0.001**

Note: Group 1:biallelic missense; Group 2:including biallelic truncating or missense plus truncating genotype; *the number of N depends on whether the reported literature can confirm the presence or absence of the phenotype

retardation, and reduced motor activity [12]. The underline mechanism is that the absence of CLPP may increase levels of CLPX protein and other mitochondrial chaperones, disrupt regulation of nuclear-encoded genes depending on the tissue, elevate mitochondrial DNA levels leading to measurable respiratory deficits, and significantly activate T cells in the spleen. Currently reported disease-associated mouse models are KO mice, primarily simulating severe disease phenotypes, some of which are similar to patient phenotypes. However, neurological phenotypes may be overlooked in these observations. Based on our genotype-phenotype analysis, we found that biallelic missense genotype variants tends to result in milder phenotypes. Therefore, future research should focus on creating knock-in mouse models to mimic these milder phenotypes. Furthermore, it is essential to further investigate in animal models whether the genotype-phenotype relationships hold true, which will guide the evaluation of CI surgery outcomes and clinical genetic counseling.

Conclusions

In conclusion, this study has expanded the understanding of *CLPP* variants in Perrault syndrome type 3 by identifying a novel compound heterozygous variant and another case with a novel missense variant along with a large deletion in *CLPP*. The findings from previous studies, combined with our results, enhance our understanding of the relationship between *CLPP* genotypes and phenotypes. Our study provides further evidence supporting the correlation between genotype and phenotype in individuals affected by Perrault syndrome type 3. These findings have important implications for genetic counseling, carrier screening, and prenatal genetic diagnosis in patients diagnosed with Perrault syndrome type 3. By offering new insights into the diagnosis and genetic counseling of Perrault syndrome type 3, this study contributes to the advancement of clinical practice in this field.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-025-00762-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Author contributions

The work was conceived, supervised the execution, acquired funding, and managed the project by H.Y. and Y.L.; Data acquisition and analysis were assisted by B.Y., X.L., X.W., and W.P.; The experimental design was completed by W.W., M.L., and X.L.; W.X. was responsible for the follow-up of case data; The manuscript was drafted by X.L.; the manuscript was revised and approved by all authors.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval for this study was granted by the Institutional Review Board of West China Hospital of Sichuan University (2021-190). Informed consent was obtained from all participants or their legal guardians.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the images in Supplementary Fig. 2.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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