Two Novel Likely Pathogenic Variants of HARS2 Identified in a Chinese Family With Perrault Syndrome

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Brief report

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Abstract

HARS2 is one of the genetic causes of Perrault syndrome, characterized by sensorineural hearing loss (SNHL) and ovarian dysfunction. Here, we identified two novel putative pathogenic variants of HARS2 in a Perrault syndrome pedigree including two affected male siblings, c.349G>A (p.Asp117Asn) and c.908T>C (p.Leu303Pro), through targeted next-generation sequencing methods. The two affected siblings (13 and 11 years old) presented with early-onset, rapidly progressive SNHL. The affected siblings did not have any inner ear malformations or delays in gross motor development. Combined with preexisting clinical reports, these findings further expanded the existing spectrum of HARS2 variants and Perrault syndrome phenotypes, which will assist in molecular diagnosis and genetic counselling of patients with Perrault syndrome.

Introduction

The HARS2 gene is mapped to chromosome 5q31.3, which contains 13 exons and spans approximately 7.9 kb. The HARS2 gene encodes the highly conserved mitochondrial histidyl-tRNA synthetase, which is involved in mitochondrial protein translation[1, 2]. In 2011, HARS2 was first identified as a cause of Perrault syndrome by genome-wide linkage analysis and candidate gene sequencing [1]. Perrault syndrome is an autosomal recessive disorder with main clinical features of bilateral SNHL, a mild to profound degree of hearing loss, and ovarian dysgenesis in females. When the onset of moderate SNHL is in early childhood, it may present as progressive hearing loss. Ovarian dysfunction ranges from primary amenorrhea to primary ovarian insufficiency (POI), which can lead to infertility. Affected males, on the other hand, show normal pubertal development and are typically fertile[3].

Diagnosis of Perrault syndrome is often based on common clinical manifestations of SNHL in females and males as well as ovarian dysfunction in females with normal karyotypes [1, 4]. Further, this diagnosis should only be made after exclusion of other potential diagnoses with symptoms similar to those of Perrault syndrome. Perrault syndrome is uncommon, and approximately 100 affected individuals have been reported to date [4]. The disease is clinically and genetically heterogeneous, and some patients show neurological signs in addition to typical deafness and premature ovarian failure [4]. Due to the complex clinical phenotype of the disease, affected males without affected sisters are diagnosed with nonsyndromic deafness rather than Perrault syndrome. The diagnosis of Perrault syndrome is confirmed by the presence of biallelic pathogenic variants in one of six genes, such as CLPP, ERAL1, HARS2, HSD17B4, LARS2, and TWWN1[1, 5–9]. Currently, these genes explain approximately 40% of the causes of Perrault syndrome, but the genetic bases of more than half the cases of Perrault syndrome remain unclear [4].

Only seventeen cases of HARS2 variants spanning nine families have been reported to date[1, 4, 10–12]. Due to its very low incidence and clinical heterogeneity, much information remains to be collected with regard to this variant. In the present study, two novel putative pathogenic variants of HARS2 were identified in two male individuals, from the same Chinese family, with autosomal recessive non-syndromic SNHL. These two cases and a review of the literature were used to explore the correlation between phenotype and the HARS2 genotype.

Materials And Methods

Patients

A two-generation Chinese family with SNHL was recruited for the present study. This family consisted of four members, including two siblings (II-1 and II-2, Fig. 1a) showing rapidly progressive prelingual SNHL and two normal parents (I-1 and I-2, Fig. 1a). A detailed physical examination and an audiometric assessment were performed on the two affected brothers, including ear electron microscopy, auditory brainstem response (ABR) testing, pure tone audiometry (PTA), computed tomography (CT) of the tympanic membrane and the mastoid process, and magnetic resonance imaging (MRI) of the bilateral inner ear canal and the brain. These tests were used to detect external auditory canal, middle ear, and inner ear malformations as well as auditory nerve abnormalities. The participating parents were assessed through clinical interviews.

Targeted next-generation sequencing and data analysis

Genomic DNA was extracted from the subjects’ peripheral blood leukocytes using a Qiagen DNA Blood Kit (Qiagen, Germany) in accordance with the standard extraction protocol, and DNA integrity was detected by agar gel electrophoresis. The target regions of 139 disease-related genes in the patients’ genomic DNA were captured using an Agilent Capture Kit. An Illumina HiSeq sequencing system (Illumina, Inc., San Diego, CA, USA) was used for Next-generation sequencing of coding regions, gene regulatory regions, and 10 bp flanking introns of target genes (Additional Table s1). The deafness-related sites of the patients’ mitochondrial DNA were detected by Matrix-assisted laser Desorption Ionization-time Of Flight Mass Spectrometry (MALDI-TOF-MS), and the candidate variant sites of the family DNA samples were verified by Sanger sequencing. The sequencing data was analyzed using the Sentience software suite, while variant annotation and screening were performed using Woxi NextCODE software (Shanghai, China).

Three major databases containing reported or potential pathogenic variants, including ClinVar, OMIM, and HGMD, were used to screen for known pathogenic variants. A population-based database of large-scale sequencing (gnomAD; http://gnomad.broadinstitute.org), the Exome Aggregation Consortium (ExAC), 1,000 genomes, and an internal whole exome sequencing (WES) database of 2,114 Han Chinese were used to filter minor allele frequencies (MAFs) <1% of the variation. Further screening for rare variants was conducted according to the interpretive guidelines of the American College of Medical Genetics and Genomics. Phenotypes of selected genes were analyzed to exclude genes not related to the patients’ clinical presentations and genetic patterns.

Validation of variants and inheritance analysis

All candidate pathogenic variants were confirmed by Sanger sequencing. Specific primers were designed to amplify the regions containing the variants by polymerase chain reaction (PCR, Additional Table s2). The PCR products were sequenced on an ABI 3730XL Genetic Analyzer (Applied Biosystems Life
Technologies) according to the manufacturer's protocols.

**In silico analysis**

A variety of tools were used to predict the pathogenicity of missense variants, including SIFT, Poly-Phen2, the Combined Annotation Dependent Depletion (CADDv1.3) score, and MutationTaster. Multiple amino acid sequence alignments of different species were conducted using ClustalW; these alignments included HARS2 orthologues from Homo sapiens, Pan troglodytes, Macaca mulatta, Cats, Mice, Zebras, Fruitflies, Caenorhabditis elegans, bovines, and Rattus as well as the human HARS paralogue. The GERP ++ score was used to evaluate evolutionary conservatism, and its value ranged from ~ 12.3 to 6.17, with 6.17 as the most conservative score [13]. To investigate the potential effects of each HARS2 variant on protein conformation, the protein structure of the HARS2 variant was predicted using the online protein model prediction server SWISS-MODEL. Analysis of protein structure changes and amino acid interactions were performed using the PyMOL Molecular Graphics System.

**Results**

**Clinical presentation**

The recruited family included two affected siblings and two parents with normal phenotypes (Fig. 1a). The proband (I-2) had not passed the Universal Newborn Hearing Screening (UNHS) and presented with mild to moderate hearing loss at one year of age. At three years of age, he was diagnosed with otitis media and was treated with a bilateral tympanostomy tube. Hearing aids were provided at the age of four years. After the initial positive effects of hearing aids, hearing in both ears decreased with each year. When the proband was 10 years old, the audiogram showed severe bilateral SNHL(Fig. 1c), and he received a cochlear implant in his right ear. The older brother of the proband (I-1) was diagnosed with bilateral profound SNHL at the age of 2.5 years (Fig. 1d) but had previously been suspected by his parents of having a poor response to sound. He had begun to use hearing aid in both ears, which initially improved his hearing but gradually lost its effect. Therefore, he received a bilateral cochlear implant at the age of seven years. The affected siblings underwent normal motor, cognitive, and behavioral development.

The patients' Romberg tests were negative, and their tandem gaits were normal. An MRI of the temporal bone excluded inner ear malformations in the affected individuals. Their parents had normal hearing and were not consanguineous. A medical examination of all the family members revealed no signs of systemic disease or malformation. A survey of this family revealed no history of deafness. The inheritance pattern of the family appeared to be autosomal recessive.

**Genetic findings**

The genomic DNA of the proband was analyzed using targeted next-generation sequencing. Two novel potentially pathogenic missense variants of HARS2—NM_012208.3:c.349G > A (p.Asp117Asn) and c.908T > C (p.Leu303Pro)—were identified. Sanger sequencing revealed that both affected siblings carried two novel missense variants of the HARS2 gene. Sanger sequencing confirmed that the father (I-1) was a heterozygous carrier of c.908T > C (p.Leu303Pro) and the mother (I-2) was a heterozygous carrier of c.349G > A (p.Asp117Asn), indicating complete cosegregation of the variants with the disease phenotype in this family (Fig. 1b).

*In silico analysis of the variants detected in HARS2*

The c.349G > A substitution results in the substitution of aspartic acid by asparagine (p.Asp117Asn). The c.908T > C results in a single amino acid substitution: leucine to proline (p.Leu303Pro). Neither of these variants were found in the ExAC or in-house WES databases, but they were found in the heterozygous state from gnomAD (Table 1). The multiple amino acid sequence alignment of HARS2 showed that the two mutant residue sites were evolutionarily conserved in nine homologous species (Fig. 2a). Both variants were located in the catalytic domain of HARS2, in accordance with previously reported potential pathogenic variants of HARS2 (Fig. 2b). These two variants were predicted to be deleterious by most of the in silico software programs (CADD, Poly-Phen2, MutationTaster, and SIFT), further supporting variant pathogenicity (Table 1).
Table 1

| ID    | mutation                           | gnomAD | CADD  | SIFT     | Polyphen-2 | Mutationtster | GERP++       | PathogeniClassicated |
|-------|------------------------------------|--------|-------|----------|------------|---------------|--------------|----------------------|
| 1     | c.72C>A (p. Cys24Stop)             | 0      | 24    | Deleterious (0) | Damaging (1) | Disease Causing (1) | Yes(5.27) | Likely Pathogeni    |
| 2     | c.137T>A (p.Leu46Gln)              | 0      | 23.8  | Tolerated (0.1) | Damaging (1.0) | Disease Causing (1) | Yes(4.07) | Uncertain Significan |
| 3     | c.259C>T (p.Arg87Cys)              | 0.00001| 31    | Deleterious (0) | Damaging (0.94) | Disease Causing (1) | Yes(5.3)  | Likely Pathogeni   |
| 4     | c.349G>A (p.Asp117Asn)             | 0.0000397| 23.9 | Tolerated (0.12) | Benign(0.186) | Disease Causing (1) | Yes(5.65) | Likely Pathogeni   |
| 5     | c.413G>A (p.Arg138His)             | 0.000028| 34    | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(6.17) | Likely Pathogeni   |
| 6     | c.448C>T (p.Arg150Cys)             | 0.0005 | 32    | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(5.3)  | Likely Pathogeni   |
| 7     | c.598C>G (p.Leu200Val)             | 0.0003 | 26.7  | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(4.38) | Likely Pathogeni   |
| 8     | c.647G>A (p.Leu216Gln)             | 0.00000797| 23.4 | Tolerated (0.15) | Damaging(0.989) | Disease Causing (1) | Yes(5.18) | Uncertain Significan |
| 9     | c.697C>T (p.Arg233Cys)             | 0      | 33    | Deleterious(0) | Damaging(0.999) | Disease Causing (1) | Yes(5.3)  | Uncertain Significan |
| 10    | c.828delTinsGTATCCCTAGTATTCTACTA   | (p.Gly277TyrfsTer3) | 0      | /      | /        | /              | /            | Likely Pathogeni    |
| 11    | c.908T>C (p.Leu303Pro)             | 0.000008| 24.2  | Deleterious (0) | Benign(0.047) | Disease Causing (1) | Yes(5.78) | Uncertain Significan |
| 12    | c.980G>A (p.Arg327Gln)             | 0.000004| 34    | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(3.95) | Likely Pathogeni   |
| 13    | c.1010A>G (p.Tyr337Cys)            | 0.000004| 28.3  | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(5.67) | Uncertain Significan |
| 14    | c.1102G>T (p.Val368Leu)            | 0.000008| 25.9  | Deleterious (0) | Damaging (0.99) | Disease Causing (1) | Yes(5.67) | Likely Pathogeni   |
| 15    | c.1439G>A (p.Arg480His)            | 0.000074| 35    | Deleterious (0) | Damaging (1)  | Disease Causing (1) | Yes(5.5)  | Likely pathogeni    |

HARS2 NM_012208.3. Output prediction of each tool-CADD defined scores ranging from 1 to 99 based on the rank of each variant relative to all possible 8.6 billion substitutions in the human reference genome. Reference genome single nucleotide variants at the top 10% of CADD scores are assigned to CADD Pathogenic, likely pathogenic, or uncertain significance, likely benign, and benign.

A homologous HARS2 model was built by SWISS-MODEL (Fig. 2c). The results showed that Asp117 residue was located in the s1 loop at the dimer interface and formed a hydrogen bond with the Lys118 residue on another monomer. The substitution of the Asp residue with an Asn residue increased interaction with the Gly277 residue (Fig. 2d). The Leu303 residue was predicted to be located in an a-helix, and the substitution of Pro residues with Leu residues lost the hydrogen bond between the Leu303 residues and the Gly297 residues; this may have affected the stability of crystal conformation (Fig. 2e).

Discussion

In the present study, we identified two novel missense variants—c.349G>A (p.Asp117Asn) and c.908T>C (p.Leu303Pro)—in the HARS2 genes of two male individuals with NSHL from the same Chinese family. Both p.Asp117Asn and p.Leu303Pro are highly conserved across species and located in the catalytic domain, which is the critical domain of mitochondrial histidyl tRNA synthetase. These two variants were found at extremely low frequencies in the control databases. Therefore, in accordance with the guidelines of the American College of Medical Genetics and Genomics for sequence variant interpretation [14], c.349G>A (p.Asp117Asn) and c.908T>C (p.Leu303Pro) were interpreted as likely pathogenic variants. Since 2011, 17 variants of HARS2 have been associated with deafness or Perrault syndrome, including c.908T>C (p.Leu303Pro) and c.349G>A (p.Asp117Asn) in the present study. The HARS2 protein is a homodimer enzyme belonging to the class II family of aminocyl-tRNA synthetases. HARS2 protein prediction consists of two domains: (1) an N-terminal catalytic domain, including a dimer interface and a HisRS-specific helical domain that binds to the acceptor stem of tRNA; and (2) a C-terminal domain, which is involved in recognition of the anticodon stem and loop of tRNA[1, 15]. Sixteen variants are located in the catalytic domain of HARS2, except for the variants, p.R480H in the domain of the C-terminal anticodon binding domain (Fig. 2b).

Individuals with Perrault syndrome have been reported both with and without neurologic features[3, 5, 7]. Some patients have shown additional clinical features, but a consistent pattern has been observed for these features, even within families. In the literature, all HARS2 families except for one reported no neurological symptoms (Table 2). Demain et al. [11] reported soft neurological features in two affected brothers in a family. One reported difficulty with fine
motor movements, while the other reported tightness in the muscles of his lower limbs. The occurrence of neurological symptoms is consistent with that observed among patients with other mitochondrial minoacyl-synthetase gene disorders, such as variants in $\text{NARS2}$ associated with non-syndromic hearing loss or Leigh syndrome\cite{16}, variants in $\text{LARS2}$, which can lead to hearing loss with or without additional neurological disease\cite{8, 17}. However, the symptoms of the two brothers in this study were not consistent. It is unclear whether the presence of neurological symptoms may be due to the clinical heterogeneity of disease caused by variants in $\text{HARS2}$.

Table 2. Clinical features of all reported patients with $\text{HARS2}$ variants.

| Patient | Origin/Descent | HARS2 Variants | Sex | Age of Onset | Age at Exam | Degree of hearing loss | Hearing habilitation | Gonadal dysfunction | Age at POI | Neurological features |
|---------|----------------|----------------|-----|--------------|-------------|------------------------|---------------------|---------------------|-----------|----------------------|
| Family 1 |                |                | F   | 34           | NR          | Mild                   | NR                  | ovarian dysgenesis, with amenorrhea and streak gonads | NR        | NO                   |
|         | European       | c.598C > G     | p.Leu200Val | c.1102G>T   | p.Val368Leu |                        |                     |                     |           |                      |
|         |                | variants      | M1   |             |             |                        |                     |                     |           |                      |
|         |                |                | M2   |             |             |                        |                     |                     |           |                      |
|         |                |                | M    | 6           | 22          | Moderate to severe     | NR                  | NA                  | NA        | NO                   |
|         |                |                | F    | 12          | 19          | Mild to moderate       | NR                  | ovarian dysgenesis, with amenorrhea and streak gonads | NR        | NO                   |
| Family 2 |                |                | F    | <3          | NR          | Profound               | NR                  | Secondary Amenorrhea | 25        | NO                   |
|         | Morocco        | c.1010A>G      | p.Tyr337Cys | Hom       | p.Tyr337Cys   |                        |                     |                     |           |                      |
|         |                | variants      | M    |             |             |                        |                     |                     |           |                      |
|         |                |                | M    |             |             |                        |                     |                     |           |                      |
|         |                |                | F    | <3          | NR          | Profound               | NR                  | Secondary Amenorrhea | 26        | NO                   |
| Family 3 |                |                | M    | NR          | NR          | NR                     | NA                  | NA                  | NO        | NO                   |
|         | proband Chinese Han |                | c.647G>A  | p.Arg216Gln |           |                        |                     |                     |           |                      |
|         |                | variants      | F    |             |             |                        |                     |                     |           |                      |
|         |                |                | M    |             |             |                        |                     |                     |           |                      |

Table 2. (Continued).
Table 2. (Continued).

| Patient | Origin/ Descent | HARS2 Mutation | Sex | Age of Onset | Age at Exam | Degree of hearing loss | Hearing habilitation | Gonad dysfun |
|---------|-----------------|----------------|-----|--------------|-------------|------------------------|---------------------|---------------|
| Family 8 | North American | c.828delTinsGTATCCCTAGTATTTCTACTA | M   | 2.5          | 14          | Severe to profound     | CI+HA               | NA            |
|         |                 | p.Gly277TyrfsTer3 |     |              |             |                        |                     |               |
|         |                 | c.1439G>A p.Arg480His |     |              |             |                        |                     |               |
|         |                 | c.1439G>A p.Arg480His |     |              |             |                        |                     |               |
|         |                 | c.908T>C p.Leu303Pro |     |              |             |                        |                     |               |
| Family 9 | North American | c.72C>A p. Cys24Stop | M   | 1.5          | 4           | Profound               | Not recorded         | NA            |
| Family 10 | Chinese Han | c.349G>A p.Asp117Asn | M   | 2.5          | 7           | Profound               | HA+Right CI          | NA            |
|         |                 | c.908T>C p.Leu303Pro |     |              |             |                        |                     |               |

HA, hearing aid; CI, cochlear implant; POI, primary ovarian insucieency; F, female; M, male; NA, not applicable; NR, not recorded.

The typical clinical phenotype of Perrault syndrome was observed in four families, and there was significant clinical heterogeneity not only between these families but also within individual family members (Table 2). Clinical features of Perrault syndrome include early or late onset of mild to profound bilateral sensorineural deafness as well as irregular menstruation, secondary amenorrhea, premature ovarian failure, and other manifestations of ovarian dysfunction in women. It is worth noting that the two female patients in the present study exhibited normal levels of luteinizing hormone, estradiol, inhibin B, and anti-Mullerian hormone and showed no signs of ovarian dysfunction; these findings may be attributed to their young age. For clinicians, sporadic males, and preadolescent females with no signs of POI, the definitive diagnosis of Perrault syndrome based on clinical features alone is a challenge[3]. It is not clear whether HARS2 is responsible for hearing loss in young women and other preadolescent women without any effect on future ovarian function; subsequent follow-up with women exhibiting HARS2 is critical to clarify this issue.
Previous studies have shown that HARS2 variants do not affect protein expression but significantly impact enzyme activity, resulting in decreased levels of aminoacylated tRNA His and further mitochondrial dysfunction, including reduced ATP synthesis due to respiratory defects, decreased mitochondrial membrane potential, impaired oxidative phosphorylation, and increased ROS. The variant of HARS2 may result in mitochondrial dysfunction by reducing enzyme activity. HARS2 is highly expressed in the cochlea and the neural tube [18], and the cochlea is particularly sensitive to mitochondrial dysfunction due to the high expression and wide distribution of this gene. Further functional experiments are needed to elucidate the pathogenesis of HARS2 variants.

In conclusion, we described a rare pedigree of Perrault syndrome. Two novel putative pathogenic variants of HARS2 were identified in two affected male individuals. These novel variants further expanded the existing spectrum of HARS2 variants and phenotypes of Perrault syndrome, which can assist in molecular diagnosis and genetic counselling of patients with Perrault syndrome. The correlation between phenotype and HARS2 genotype was explored alongside preexisting clinical reports. The present cohort was too small to elucidate a relation between genotypes and phenotypes, as 17 variants of HARS2 have been associated with deafness or Perrault syndrome in 19 cases to date. A series of studies will be performed in the future to explore phenotype-genotype correlations within a larger sample.

**Abbreviations**

SNHL: sensorineural hearing loss; POI: primary ovarian insufficiency

**Declarations**

**Authors’ contributions**

All authors participated in the design of the case report and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Boards of Sichuan Provincial People's Hospital.

**Consent for publication**

Written informed consent was provided by the patient's parents.

**Competing Interests**

The authors declare that no conflict of interest to report.

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Figure 1

Pedigree and sequence analysis of an NSHL family. (A) The pedigree of the family. Affected individuals are denoted in black. The arrow indicates the proband. W, wild type; M1, HARS2 gene NM_012208.3;c.349G>A (p.Asp117Asn); M2, HARS2 gene NM_012208.3;c.908T>C (p.Leu303Pro). (B) Sanger sequencing electropherograms of two variants. (C) Audiogram of the proband at 10 years of age. (D) Audiogram of the affected brother at 2.5 years of age.
In silico analysis of HARS2 variants. (A) Multispecies sequence alignment of the HARS2 protein showing the evolutionary conservation of two mutated amino acids (p.Asp117Asn and p.Leu303Pro). Red shading indicates amino acids shared among all eight species. (B) Newly identified and previously reported HARS2 variants are marked along the schematic representation of HARS2. The N-terminal catalytic domain (amino acid; aa 1–405) and the C-terminal anticodon binding domain (aa 406–506). The catalytic domain contains the highly conserved dimer interface shown in dark blue (aa 65–177) and the histidine recognition and binding sites shown in pink (HisA: aa 327–332 and HisB: aa 361–365) (C) Crystal-structure-based in silico modeling of a dimeric HARS2 protein. This 3D model of the HARS2 protein is based on the structure of dimeric human HisRS (PDB ID 4phc). Two monomers of the homodimer are shown in green and blue, respectively. The Asp117 residue is located in the s1 loop at the dimer interface, as indicated by the red stick. The Leu303 residue is...
located in an α-helix displayed in orange. (D) Close-up view of residue 117 and other residues that interacted. (E) Close-up view of residue 303 and other residues that interacted.

**Supplementary Files**

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