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Exome analysis identified a novel missense mutation in the *CLPP* gene in a consanguineous Saudi family expanding the clinical spectrum of Perrault Syndrome type-3

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ABSTRACT

Perrault syndrome (PRLTS) is a clinically and genetically heterogeneous disorder. Both male and female patients suffer from sensory neuronal hearing loss in early childhood, and female patients are characterized by premature ovarian failure and infertility after puberty. Clinical diagnosis may not be possible in early life, because key features of PRLTS, for example infertility and premature ovarian failure, do not appear before puberty. Limb spasticity, muscle weakness, and intellectual disability have also been observed in PRLTS patients. Mutations in five genes, *HSD17B4*, *HARS2*, *CLPP*, *LARS2*, and *C10orf2*, have been reported in five subtypes of PRLTS. We discovered a consanguineous Saudi family with the PRLTS3 phenotype showing an autosomal recessive mode of inheritance. The patients had developed profound hearing loss, brain atrophy, and lower limb spasticity in early childhood. For molecular diagnosis, we complimented genome-wide homozygosity mapping with whole exome sequencing analyses and identified a novel homozygous mutation in exon 6 of *CLPP* at chromosome 19p13.3. To our knowledge, early onset with regression is a unique feature of these PRLTS patients that has not been reported so far. This study broadens the clinical spectrum of PRLTS3.

1. Introduction

Perrault syndrome (PRLTS) is an autosomal recessive disorder characterized by sensory neuronal hearing loss (SNHL) and premature ovarian failure secondary to ovarian dysgenesis [1]. Additional clinical features including spasticity, peripheral neuropathy, ataxia, and learning difficulty have also been described [2–4]. SNHL and limb spasticity may start in early life with gradual progression and variable severity leading to complete deafness and wheelchair dependency or inability to walk [5,6]. The infertility features, defined by impaired sex hormone profiles and premature menopause can only be detected after puberty [2,3]. The phenotypic variability and age-dependent presentation of PRLTS in such patients can pose a diagnostic challenge in clinical practices [2,7].

PRLTS has been classified into five subclasses on the basis of causative genes.

PRLTS type-1 (PRLTS1, OMIM 233400) patients are characterized by hearing loss, ovarian dysgenesis leading to female infertility, and markedly reduced D-bifunctional protein (DBP) enzymatic activity causing male infertility, ataxia, and peripheral neuropathy [6,8,9]. Mutations in a gene on chromosome 5q23.1, encoding a multifunctional peroxisomal enzyme 17β-hydroxysteroid dehydrogenase type 4 (*HSD17B4*, OMIM 601860) cause PRLTS1 [6,8,9]. The HSD17B4 enzyme is also known as DBP and is involved in fatty acid β-oxidation and steroid metabolism [10].

PRLTS type-2 (PRLTS2, OMIM 614926) patients are characterized by SNHL in both males and females, and gonadal dysgenesis in female patients only [11]. Mutations in the mitochondrial histidyl-tRNA synthetase (*HARS2*, OMIM 600783) gene on chromosome 5q31.3 cause PRLTS2 because of the reduction of HARS2 enzyme activity, which

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leads to defective mitochondrial protein synthesis and results in mammalian gonadal dysgenesis [11].

PRLTS type-3 (PRLTS3, OMIM 614129) patients are characterized by progressive hearing loss, female infertility and premature menopause secondary to ovarian dysgenesis, microcephaly, epilepsy, and growth and mental retardation because of pathogenic variations in the caseinolytic mitochondrial matrix peptidase proteolytic subunit (*CLPP*, OMIM 601119) gene on chromosome 19p13.3 [12].

PRLTS type-4 (PRLTS4, OMIM 615300) patients are characterized by a severe hearing loss at higher frequencies, and premature ovarian failure, small uterus, and increased gonadotropin levels in females [13] that result from mutations in leucyl-tRNA synthetase 2 (*LARS2*, MIM 604544) gene on chromosome 3p21.31 [13]. *LARS2* encodes a mitochondrial tRNA synthetase that appears to play a critical role of in the maintenance of ovaries and hearing [13].

PRLTS type-5 (PRLTS5, OMIM 616138) patients are characterized by progressive ataxia, axonal neuropathy, hyporeflexia, abnormal eye movements, progressive hearing loss, and ovarian dysgenesis [14]. Recently, causative mutations in the twinkle primase-helicase (*C10orf2*, MIM 606075) gene on chromosome 10q24.31 have been identified as the cause of PRLTS5 [14].

Here, we present clinical and molecular investigations of a consanguineous PRLTS3 family from Saudi Arabia.

2. Materials and methods

2.1. Ethical approval

An informed written consent was signed by each participant or by the legal parents of patients prior to commencement of this study. All the participants agreed that the research outcomes could be published. The study was also approved (ref. # 24-14), according to the Declaration of Helsinki, by the Institutional Review Board of the Princess Al-Jawhara Albrahim Center of Excellence in Research of Hereditary Disorders and Unit of Biomedical Ethics Research Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. All the affected and unaffected individuals were examined thoroughly by genetic consultants at the Department of Genetic Medicine, King Abdulaziz University Hospital, Jeddah. Information regarding the disease history of the affected patients and the family consanguinity was provided by interviewing the parents.

2.2. Study subjects

The five generation family (Fig. 1) belonged to a remote village in the southwestern region of Saudi Arabia. Peripheral blood samples from eight family members including both parents were collected in K3 EDTA tubes (BD Vacutainer, USA) and stored at 4 °C. For genomic DNA isolation and quantification, we used commercially available kits (QIAamp, Qiagen, USA) and a Nanodrop-2000 spectrophotometer (Thermo Scientific, USA), respectively.

2.3. Chromosomal studies

To investigate a possible involvement of chromosomal aberrations, we performed microarray analysis with a HumanCytoSNP-12 v12.1 chip (Illumina, USA) that has 300K single nucleotide polymorphism (SNP) resolution in two affected (V-4 and V-5) individuals using iScan (Illumina) according to the manufacturers' protocol. The SNP array data was analyzed with the GenomeStudio genotyping module 5 software (Illumina) for copy number variation and homozygosity mapping.

2.4. Exome sequencing

The blood samples from the two affected individuals (V-4 and V-5) were also subjected to whole-exome sequencing using 100X paired-end-sequencing. The 51 Mb SureSelect libraries (Agilent Technologies, USA) were constructed using 2 μg genomic DNA from each individual. The target regions with an average throughput depth of 130 bp and 100 bp paired-ends reads were sequenced on a HiSeq 2000 system (Illumina, USA). Sequences were aligned using the BWA Aligner (http://bio-bwa.sourceforge.net/), and copy number variation and insertion/deletion (InDel) detection were performed using SAMTOOLS (http://samtools.sourceforge.net/). The sequence reads were mapped

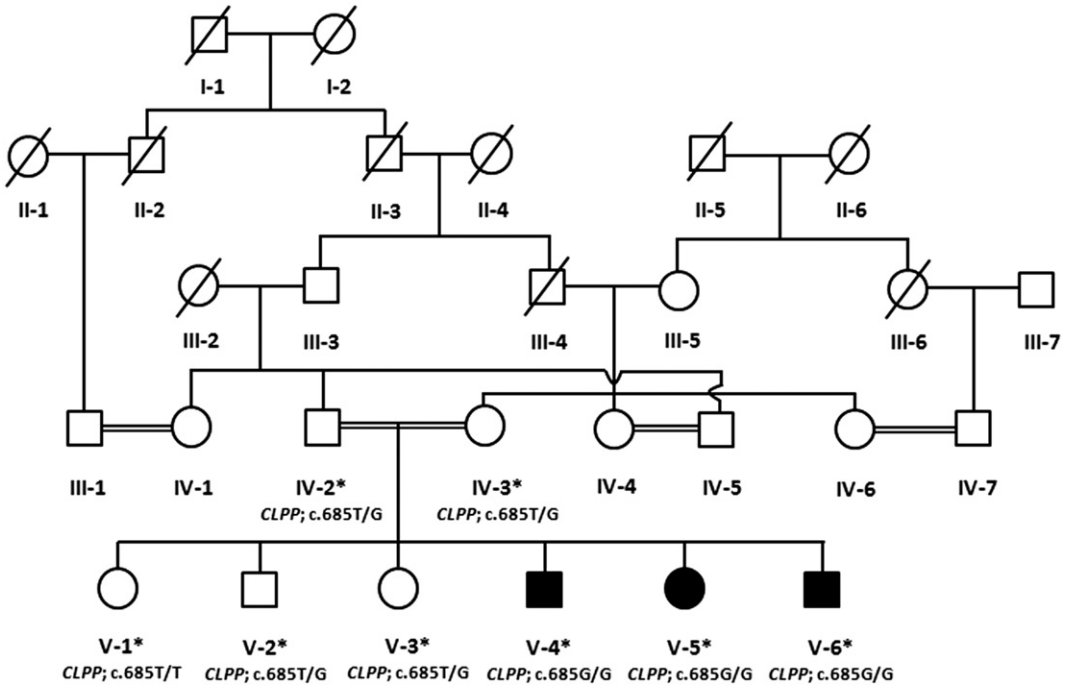

Fig. 1. Pedigree of the parents showing consanguinity and autosomal recessive mode of inheritance in affected individuals. The asterisk (*) indicates the samples that were validated by Sanger sequencing. The genotypes for the *CLPP* mutation are also shown.