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Mutations of *SGO2* and *CLDN14* collectively cause coincidental Perrault syndrome

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accuracy of the data analyses. Research concept and study design: TBF, SR, RF, AUR. Analyses and interpretation of the data: RF, AUR, PLF, RJM, LD, SZ, AAK, DT, MZA, GB, SNK, WGN.

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Conflict of interest

The authors declare no conflict of interests.

ABSTRACT

Perrault syndrome (PS) is a genetically heterogeneous disorder characterized by primary ovarian insufficiency (POI) in females and sensorineural hearing loss in males and females. In many PS subjects, causative variants have not been found in the five reported PS genes. The objective of this study was to identify the genetic cause of PS in an extended consanguineous family with six deaf individuals. Whole exome sequencing (WES) was completed on four affected members of a large family, and variants and co-segregation was confirmed by Sanger sequencing. All hearing impaired individuals, including the proband, are homozygous for a pathogenic variant of *CLDN14*, but this only explains the deafness. The PS proband is also homozygous for a frameshift variant (c.1453_1454delGA, p.Glu485Lysfs*5) in exon 7 of *SGO2* encoding shugoshin 2, which is the likely cause of her concurrent ovarian insufficiency. In mouse, *Sgol2a* encoding shugoshin-like 2a is necessary during meiosis in both sexes to maintain the integrity of the cohesin complex that tethers sister chromatids. Human *SGO2* has not previously been

implicated in any disorder, but in this case of POI and perhaps others, it is a candidate for unexplained infertility.

Key words: coincidental syndrome - cohesin - *CLDN14* - ovarian insufficiency - Perrault syndrome - *SGO2*, Shugoshin-2 - *Sgol2a*

INTRODUCTION

Perrault syndrome (PS) is a genetically heterogeneous disorder characterized clinically by sensorineural hearing loss (SNHL) in males and females and primary ovarian insufficiency (POI) (1). Individuals with PS may also manifest neurological features including peripheral neuropathy, ataxia, mild learning disability (2). Moreover, growth hormone deficiency and Marfanoid features have also been reported as part of the phenotypic spectrum of PS (3, 4). Four of the five genes (*CLPP*, MIM 614129; *HARS2*, MIM 614926; *LARS2*, MIM 615300; *C10orf2*, MIM 616138) encode proteins that function in mitochondrial proteostasis and when mutated cause Perrault syndrome. The first reported mutated gene associated with PS (*HSD17B4*, MIM 233400) encodes a peroxisomal protein (5). There are also several individuals with PS who have unresolved genetic etiologies that warrant a search for additional PS genes (6).

In this study we have identified additional genetic complexity in a PS proband of a large Pakistani family segregating deafness. The highly consanguineous pedigree includes three affected males and three affected females, only one of which has POI. We considered the possibility either that there is a single PS gene segregating with incomplete penetrance for POI or one recessive mutant gene is responsible for the hearing loss and another is independently responsible for the POI. Using whole exome sequence (WES) data we show that all of the hearing loss segregating in family PKDF063, including the proband, is attributable to a well-

characterized pathogenic homozygous missense variant of *CLDN14* (7-9). Homozygosity for a two nucleotide deletion in the protein coding exon 7 of *SGO2*, a gene hitherto not involved in any human disorder, independently is the likely cause of POI in the hearing impaired female proband.

PATIENTS AND METHODS

Family PKDF063 includes 153 individuals of which 39 individuals were consented for participation in our study. Hearing loss segregating in members of family PKDF063 was noted during their first year after birth by their mothers. Bilateral severe to profound deafness was confirmed by an audiologist. Although not an objective evaluation, parents said that their deaf children, including the PS proband, have normal intelligence and motor development.

Genomic DNA was extracted from peripheral blood leukocytes. One affected individual and the proband were initially screened by di-deoxy sequencing using BigDye (Applied Biosystems, Foster City, CA, USA) for pathogenic variants in *CLPP*, one of the five reported PS genes. We also screened for variants of *GJB2* (DFNB1A, MIM 220290) and *HGF* (DFNB39, MIM 608265) since in Pakistan mutant alleles of these two genes are common causes of deafness. WES was performed with genomic DNA from five individuals (four affected; one unaffected) of family PKDF063 (Fig. 1) using a Nextera Rapid Capture Exome kit and a HiSeq1500 instrument (Illumina San Diego, CA, USA). Computational analyses used a GATK (Genome Analysis Toolkit) pipeline followed by variant calls that were annotated with Annovar v2014_07_14. Short-listed variants were verified by di-deoxy sequencing using an ABI3730XL genetic analyzer (Applied Biosystems).

RESULTS

Clinical data

The proband (Fig. 1a, individual 140) is twenty-four years old and has a clinical diagnosis of PS (Fig. 1c) characterized by SNHL and POI. She was born to a consanguineous union and has two normal hearing and two hearing impaired siblings. Two additional branches of this pedigree include three additional deaf subjects (Fig. 1a, individuals 146, 148 and 150). The deaf proband presented at 24 years of age with a history of oligomenorrhoea. She had a delayed menarche at 17 years of age and reported irregular menstrual cycles during the succeeding few years followed by oligomenorrhoea and now menstruates once every 5-7 months. The endocrine profile of the proband revealed a low progesterone level ($<0.2\text{ng/ml}$; reference range of $0.2\text{-}1.4\text{ ng/ml}$ in the follicular stage, $4\text{ to }25\text{ ng/ml}$ luteal phase, $0.1\text{-}1$ post-menopausal), an estradiol level of 46.4pg/ml , follicle stimulating hormone (FSH) of 70.1mIU/ml that is in the reference range for post-menopausal women and a luteinizing hormone (LH) level of 37.4mIU/ml consistent with hypergonadotropic hypogonadism. The proband's pelvic ultrasound shows normal appearing ovaries and a small uterus with thin endometrial lining measuring $5.7\text{ cm} \times 2.3\text{ cm} \times 3.0\text{ cm}$ (reference size 7.6 cm length $\times 4.5\text{ cm}$ width $\times 3\text{ cm}$ thick and an endometrial thickness of 2 mm to 16 mm depending on the stage of the menstrual cycle). The proband's deaf sister (individual 139, 22 yo) has normal reproductive cycles, a hormone profile that falls within the normal reference range for her reproductive age, used a hearing aid since she was a three-year old and communicates verbally and in sign language.

Massively parallel and Sanger sequencing

Variants in two genes survived the initial filtering criteria (Table 1). The c.1664C>T variant in *SON* (rs13049658; p.(Thr555Met; NM_138927)) has an allele frequency of 14% (21 of

150 chromosomes) in Pakistani controls indicating that it is a benign polymorphism. However, the c.254T>A variant in *CLDN14* (rs74315437; p.(Val85Asp)) is a known pathogenic allele (7, 8, 11) and co-segregates with hearing loss in family PKDF063. The six individuals with bilateral severe to profound hearing loss are homozygous for c.254T>A, whereas 33 hearing individuals in this family are either heterozygous for c.254T>A or homozygous for the reference allele (Fig. 1a). Homozygous or compound heterozygous pathogenic variants of human *CLDN14* cause nonsyndromic deafness DFNB29 (MIM605608), and deaf females homozygous for c.254T>A are fertile (11). Moreover, claudin 14 null mice are fertile (9).

Next we pursued an explanation for the POI in the deaf proband (individual 140) diagnosed with PS by reanalyzing her WES data. There was no pathogenic variant in the five reported PS genes. However, using the aforementioned filtering criteria we found a frameshift variant that deserved further consideration. This variant (Fig. 1a) is a deletion in exon 7 of *SGO2* at chr2: 201,436,522_523delGA (GRCh37/hg19), c.1453_1454delGA (NM_152524.5), p.Glu485Lysfs*5 (NP_689737.4) encoding shugoshin 2 (MIM 612425; previously symbol was *SGOL2* encoding shugoshin-like 2). We then analyzed WES data for pathogenic variants of *SGO2* in five individuals with a diagnosis of PS, but lacking a molecular genetic etiology. In addition, Sanger sequencing of *SGO2* was performed in five individuals with POI of unknown etiology ascertained in the UK (6). In these ten individuals, no pathogenic variants in *SGO2* were identified.

The proband in family PKD063 is homozygous for *SGO2* p.(Glu485Lysfs*5). Her parents are carriers as are at least 14 others in this family (Fig. 1a). Variant c.1453_1454delGA is not present in 188 ethnically matched control chromosomes and is absent in the ExAC database of 121,412 chromosomes. Moreover, there are no homozygous loss of function variants of *SGO2*

reported in ExAC indicating that biallelic loss of function variants of *SGO2* are likely to result in a clinically apparent phenotype (12).

DISCUSSION

We describe the first example of an individual with a clinical diagnosis of PS that can be ascribed to a combination of homozygous variants of two unlinked genes, *CLDN14* and *SGO2* encoding shugoshin 2. *Cldn14* encodes a tight junction protein that is expressed abundantly in hair cells of the inner ear (<https://shield.hms.harvard.edu/viewgene.html?gene=Cldn14>) and is essential for the integrity of the cation-restrictive paracellular barrier of the reticular lamina in the organ of Corti (9). In the inner ear of postnatal *Cldn14*-null mice, the neurosensory outer and inner hair cells, which are responsible for sound transduction, rapidly degenerate resulting in profound deafness (9).

In mouse, *Sgol1* encodes Shugoshin-like 1, which is necessary during mitosis for chromosome segregation. The absence of meiosis-specific shugoshin-like 2a in mouse encoded by *Sgol2a* (orthologue of human *SGO2*) causes infertility in males and females resulting from the premature loss of centromeric cohesion and an inability to bi-orient at the equatorial plate (13, 14). Consequently, gametes are produced with an abnormal number of chromosomes. In elderly human oocytes, a reduced level of shugoshin 2 was suggested to have a role in premature separation of dyads resulting in aneuploidy (15). Recently, frameshift variants of *STAG3*, also necessary for sister chromatid cohesion and synapsis, were associated with infertility in two consanguineous families (16, 17). Additionally, *Stag3* null female and male mice are sterile, an observation that further strengthens the argument for the association of pathogenic variants of genes encoding the other members of the cohesin ring complex in POI and infertility (16).

Hearing loss and infertility are both genetically heterogeneous human disorders (18). In retrospect, given the large number of genes necessary for normal hearing and independently for fertility (19), it is not surprising that a person diagnosed clinically with Perrault syndrome would have biallelic pathogenic variants of two different genes, potentially assorting and acting independently. The proband of family PKDF063 (Fig. 1) is not masquerading as a PS subject. PS is her diagnosis and it is not an example of digenic inheritance where variants of two genes acting in concert explain a phenotype (20). The term “blended” has been suggested for the independent contributions to phenotype of two or more genes (21), but “blended” calls to mind a discredited nineteenth century notion of “blending inheritance”. In 1979, John M. Opitz suggested “coincidental syndrome” to describe the co-occurrence of more than one etiological distinct disorder in a patient. In the case described here, the proband manifests two genetically distinct disorders, deafness and POI, that co-occur by chance and yet are the clinical hallmarks of PS (22).

In summary, we observed that homozygosity for a rare protein-truncating DNA variant of *SGO2* as the likely cause of POI and in combination with a reported pathogenic recessive variant of *CLDN14* associated with SNHL coincidentally causes PS. Coincidence may explain undetermined genetic underpinning in other subjects with definitive audiological and hormonal findings typical of PS and the presumption of a single etiology. Finally, among several genes necessary for fertility (23), our data indicate that unexplained non-syndromic infertility or subfertility may also result from pathogenic variants of *SGO2*.

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Legends

Fig. 1 Pedigree, clinical data and molecular genetics of family PKDF063. (a) The proband with Perrault syndrome is indicated by an arrow. She has normal breast development (Tanner stage 5), secondary sexual hair and no evidence of cognitive impairment, sensory or motor peripheral neuropathy or Marfanoid features. Genotypes of *CLDN14* and *SGO2* for each of the ascertained 39 individuals are provided beneath their symbols. Individual 96 developed secondary amenorrhea of unknown etiology after the birth of her first daughter. Her serum levels were LH of 5.80 IU/L, FSH of 6.56 IU/L and estradiol of 770 pg/ml, indicative of hypogonadotropic

hypogonadism suggesting a pituitary etiology acquired later in life. Squares and circles represent male and female individuals, respectively and numbers inside squares and circles are additional siblings. (b) Gene structure of *SGO2* along with the location of a frameshift deletion in exon 7. Horizontal lines represent introns whereas thin and thick bars represent untranslated regions and exons, respectively. DNA chromatogram showing the missense variant of *CLDN14* and a novel frameshift deletion of *SGO2*. The sites of the two variants are denoted by semitransparent gray rectangles. (c) Hormonal profiles of individuals 139 (22 yo) and 140 (23 yo) and the normal reference ranges. A raised FSH level is a key biochemical indicator of primary ovarian insufficiency while LH can remain within the normal range or be raised. Chr; Chromosome.

Table 1. Filtering criteria to evaluate variant pathogenicity in WES data from family PKDF063

Step	Variant filtering criteria
1	Depth of ≥ 20 unique reads with no strand bias and a minimum quality score > 20 for GATK to call the variant
2	Variant is homozygous or in compound heterozygosity in the four deaf PKDF063 individuals
3	An allele frequency < 0.005 in the ExAC database
4	Absence of variant in 94 ethnically matched control individuals
5	Missense variant predicted to be pathogenic by Polyphen-2, SIFT, Mutation Taster, FATHMM and Mutationassessor followed by manual inspection of conservation of wild type residue among vertebrates