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Supplemental Data

Perrault Syndrome Is Caused by Recessive Mutations in *CLPP*, Encoding a Mitochondrial ATP-Dependent Chambered Protease

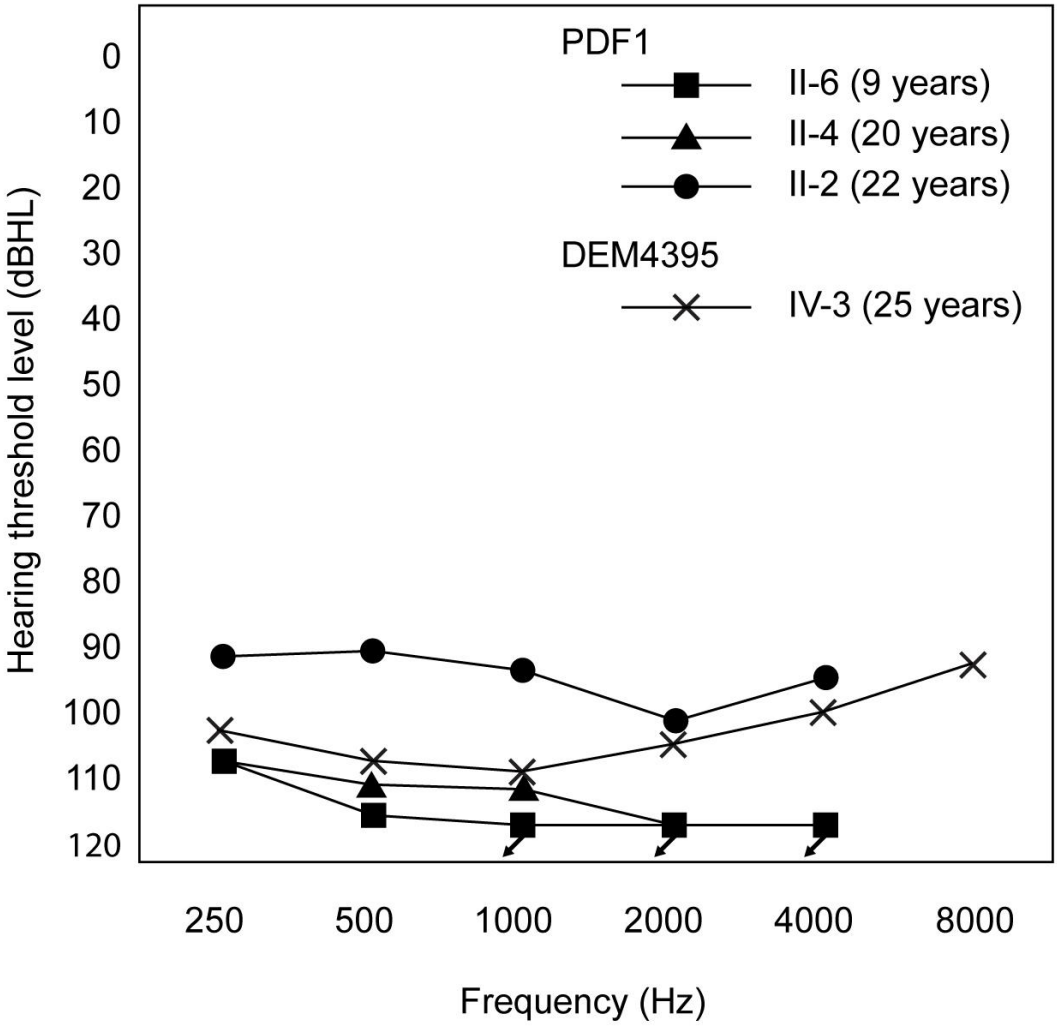

Figure S1. Audiograms of Affected Individuals from Family PDF1 and the Affected Male from Family DEM4395 Demonstrating Profound SNHL

The audiograms from the affected females in family DEM4395 were not available. Audiograms for family PKDF291 published previously.[14]

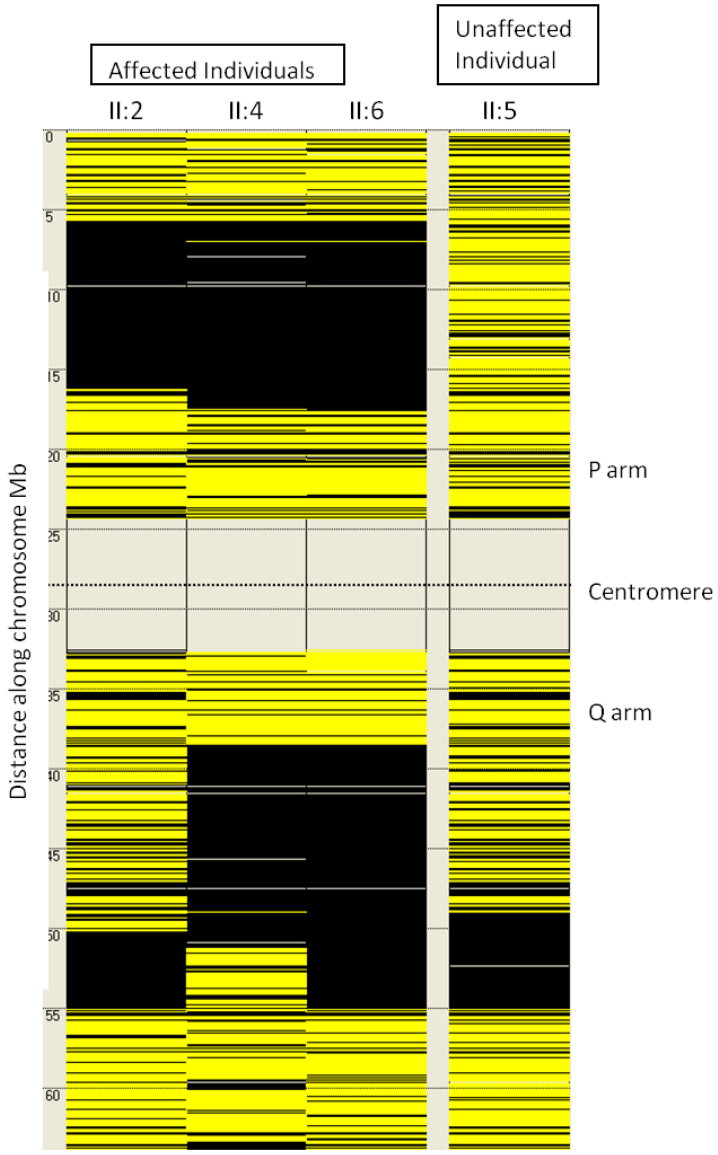

Figure S2. Mapping Data Defining Overlapping Regions of Homozygosity on the Short Arm of Chromosome 19 in Family PDF1

Affymetrix V6.0 autozygosity mapping data in AutoSNPa for three affected siblings and one unaffected sibling in family PDF1 indicating autozygous (black block) region of 10.63 Mb shared on chromosome 19p13.3-13.11.

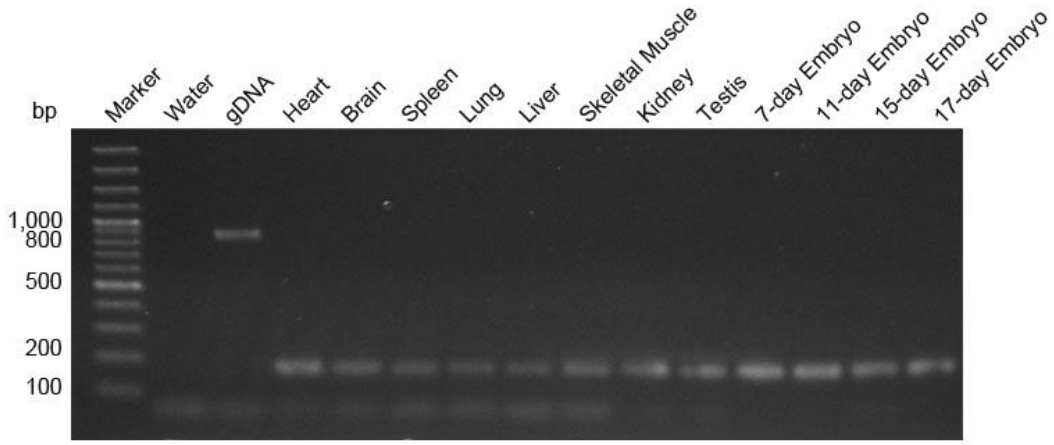

Figure S3. *Clpp* Is Expressed in 12 Mouse Tissue cDNAs Assayed by RT-PCR

RT-PCR demonstrates expression of *Clpp* in all cDNAs included in Mouse Tissue Panel I (Clontech 636745). RT-PCR reactions were performed using primers (MD081F, 5' CCCATAGTGGTGGAGCAGAC and MD081R 5' GGGCTTCTTGTTGCTTTCAG) designed to amplify a 165 bp product encoded by mouse *Clpp* exons 1, 2 and 3 (NM_017393). Using these same primers, contaminating mouse genomic *Clpp* DNA will amplify an 873 bp product. RT-PCR products were visualized on a 2% agarose gel. A 165 bp product corresponding to expression of *Clpp* mRNA was observed in adult mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, testis, and 7-day embryo, 11-day embryo, 15-day embryo, and 17-day embryo. An 873 bp product corresponding to amplification of genomic *Clpp* was observed only in the gDNA control sample. The 165 bp product was absent in the water and gDNA controls. For the RT-PCR, 1 ng of normalized mouse cDNA (Clontech) was used in a 20 uL reaction containing 1 unit of Taq DNA Polymerase (GenScript), 1X buffer containing 750 nM $MgCl_2$, 250 nM of each primer (IDT), and 200 nM dNTPs. Reaction conditions were as follows: 2 min at 95°C, 32 cycles of 95°C for 15 sec, 62°C for 15 sec, and 72°C for 10 sec, and a final extension at 72°C for 3 min.

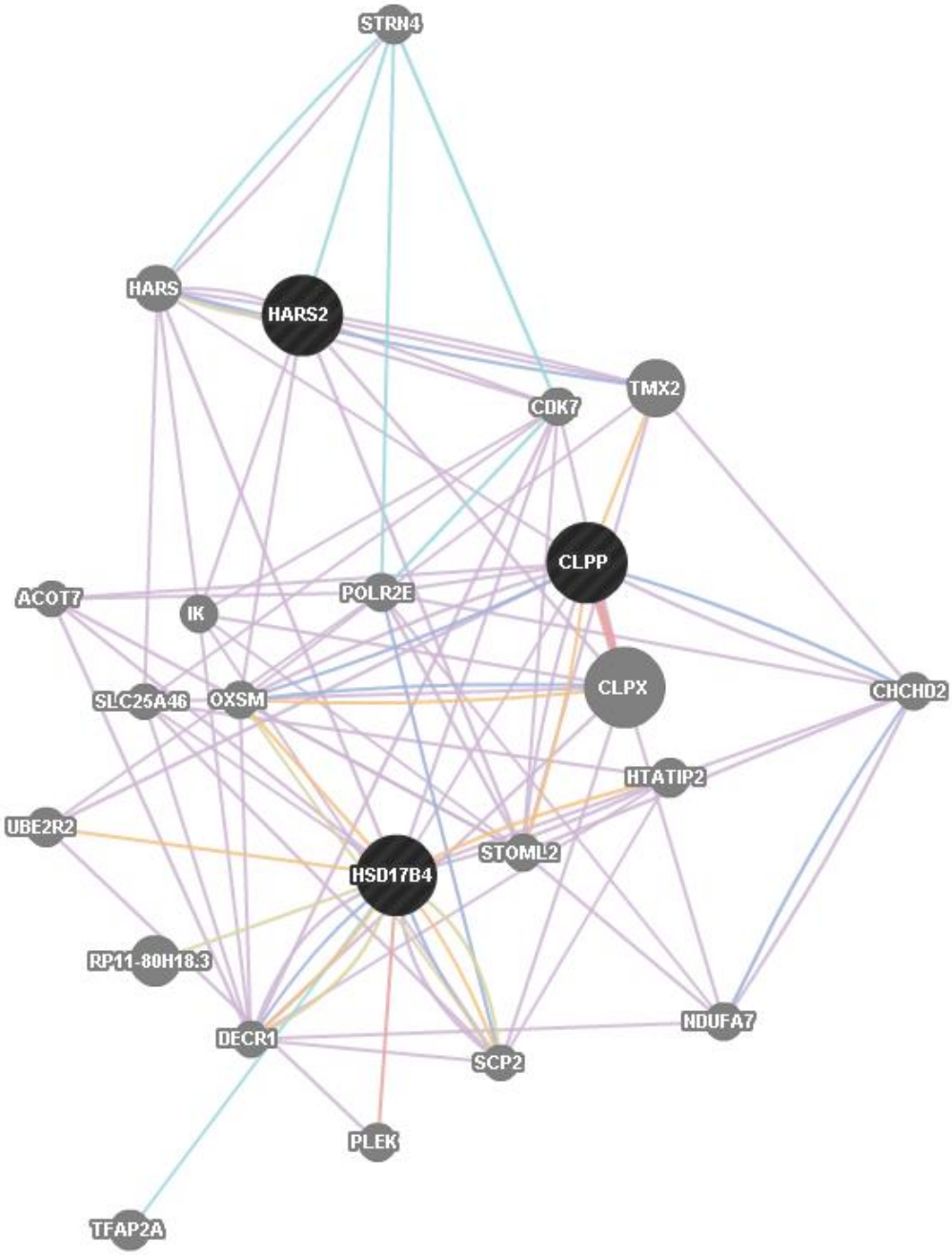

Figure S4. GeneMania Prediction of Interactions between the *HSD17B4, HARS2* and *CLPP* Genes and Their Protein Products Known to Cause Perrault Syndrome

These functional predictions are based on co-expression, co-localization and protein domain similarity.

Table S1. Mapping, Sequencing, and Gene Discovery

Family	Linked/homozygous region	Exome sequencing[a]	
		Coverage	>10x
PDF1	chr19: 5,765,869-16,392,163	120x	93%
PKDF291	chr19: 4,949,357-9,118,153	34x	72%
DEM4395	chr19: 1-15,640,972	54x	88%
Shared	chr19: 5,765,869-9,118,153		

	CLPP variant			Predicted effect[b]				Frequency in controls	
	chr19	cDNA	Effect	Polyphen-2	SIFT	MT	GERP	Pakistani	EVS[c]
PDF1	6,364,528	c.433 A>C	p.Thr145Pro	0.999; 0.994	0.00	1.000	4.17	0/193	0/6500
PKDF291	6,364,535	c.440 G>C	p.Cys147Ser	0.963; 0.869	0.00	1.000	5.18	0/483	0/6500
DEM4395	6,361,955	c.270+4A>G	aberrant splice	-	-	0.999	4.37	0/386	-

[a] Average coverage for targeted base pairs and % of base pairs with >10-fold coverage in the homozygous region of each family

[b] Polyphen-2 HumDiv and HumVar scores
SIFT P-value for probability that change is tolerated
Mutation Taster (MT) P-value for Bayesian prediction that mutation is damaging
GERP - Genomic Evolutionary Rate Profiling

[c] Exome sequences of 4300 European Americans and 2200 African Americans on the Exome Variant Server (EVS)

In family PDF1 paired-end libraries were constructed from genomic DNA extracted from blood and sonicated to approximately 200bp. After quality control, the libraries were hybridized to biotinylated DNA oligonucleotide baits from the SeqCap EZ Human Exome Library v1.0 (Roche NimbleGen), purified by streptavidin-bound magnetic beads, amplified and sequenced on a GAIIX instrument (Illumina). Four barcoded samples were multiplexed per lane. Bioinformatic analysis was carried out using the Illumina pipeline v1.6 (default parameters). In Family PKDF291 the genomic DNA of affected individual II-3 was sheared to ~ 100 bp (Covaris) and used to prepare a whole exome library. The amplified library was hybridized with whole exome probes

using Agilent SureSelect Human All Exon 50Mb kit. The library was sequenced using a SOLiD 5500 instrument (AB). The data was mapped to the human hg19 reference genome using LifeScope software (AB) and variants were annotated using wAnnovar (http://wannovar.usc.edu/). In Family DEM4395 sequence capture was performed in solution with the Roche NimbleGen SeqCap EZ Human Exome Library v2.0. The sequencing was performed using an Illumina HiSeq2000. Variant detection and genotyping were performed using various components of the Genome Analysis ToolKit.

Table S2. Splicing Assay

CLPP gDNA in pSPL3[a]	# clones sequenced	Wild type splicing of exon 2 to exon 3	Retention of intron 2	Aberrant splicing
Wild type allele	37	23	11	3
c.270+4A>G mutant allele	29	1[b]	28	0
c.270+1G>A control mutation	39	0	39	0

[a] Primers were designed to amplify a 1.3 Kb genomic DNA fragment encompassing *CLPP* exon 2-3, intron 2, and some surrounding intronic sequences. The genomic DNA of carrier individual DEM4395-1 was amplified with primer pair 5'-AATTCTGGAGCTCGAGACACGGTTCGGGGGCTCC-3' and 5'- CAGATATCTGGGATCCGCCTCCATCATAAGTGCTCAATC-3'. Fifteen nucleotide long sequences were added at the 5' ends of both primers that were homologous to the sequences at the ends of pSPL3 exon trapping vector linearized with *Xho1* and *BamH1* for cloning into pSPL3 vector using InFusion Advantage PCR Cloning Kit (Clontech). Clones were sequenced to identify a wild type allele and a c.270+4A>G mutant allele. In addition, the wild type clone was used as a template for *in vitro* site-directed mutagenesis (QuickChange Lightening Site-Directed Mutagenesis Kit, Agilent cat #210519) to obtain a c.270+1G>A control mutation, which was expected to ablate splicing at the exon 2 donor site. All clones were Sanger sequence verified. The multiple cloning sites of pSPL3 vector are located between functional splice donor and splice acceptor sites of vector exons. Splicing can occur between vector exons and wild type *CLPP* exons. COS-7 cells were transfected (Lipofectamine, Invitrogen) with each of the three coded constructs with two independent determinations. After 24 hours, total RNA was extracted with Trizol (Invitrogen), first strand cDNA was synthesized with Superscript III RT (Invitrogen) and vector specific oligo (5'-ATCTCAGTGGTATTTGTGAGC-3'). Primary and nested secondary PCR was performed with vector specific primers and products from the nested PCR were subcloned, colony purified, and individually Sanger sequenced. Because only one wild type splice product was found among 29 clones sequenced, the splicing assay was performed two more times but just with the c.270+4A>G vector to detect wild type splicing that might occur between exons 2 and 3 for this allele. Sequence analysis of a total of 20 clones revealed 13 with intron 2 retention, two clones utilizing a cryptic donor splice site (c.255_256GT) in exon 2 that spliced to exon 3, and five clones with aberrant splicing that excluded either exon 2 or exon 3 and are presumed to be artifacts.

[b] Wild type splicing was independently confirmed in additional experiments. Nested PCR splicing products of approximately 300-350 bp detected on an agarose gel indicated possible wild type splicing for the c.270+4A>G allele that we confirmed by gel purification, cloning and Sanger sequencing (Figure 2E).