PKHD1L1, a Gene Involved in the Stereociliary Coat, Causes Autosomal Recessive Non-syndromic Hearing Loss

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Shared first (*), second(**), last (***)

INTRODUCTION

- A substantial number of hearing loss-associated genes remain uncharacterized.
- Transient stereocilia coat proteins remain poorly described but facilitate key processes in the maturation of the stereocilia bundle.
- Polycystic kidney and hepatic disease 1-like 1 (PKHD1L1) is enriched at the tips of OHC stereocilia bundles and hypothesized to form the stereociliary surface coat.[1]
- Mice lacking *Pkhd1l1* displayed elevated ABR thresholds and DPOAEs[1], while zebrafish lacking *pkhd1l1a* and *pkhd1l1b* show deficient auditory startle responses.[2]
- **<u>Objective</u>: To determine if deleterious *PKHD1L1* variants cause human hearing loss**

METHODS

- Families were recruited from hearing loss cohorts from Boston Children's Hospital, University Medical Center Göttingen, Henan hearing loss cohort, and University of Punjab.
- Exome sequencing and bioinformatics analysis prioritized variants based on allele frequency, variant type, predicted deleteriousness, and evolutionary conservation.
- Missense variants identified in family 1 were introduced via site-directed mutagenesis into *Mm* PKHD1L1 protein fragments; Nanoscale differential scanning fluorimetry (NanoDSF) was employed for functional evaluation of missense variants.
- A minigene assay evaluated splicing effects of the c.1813G>A variant in family 4.

RESULTS

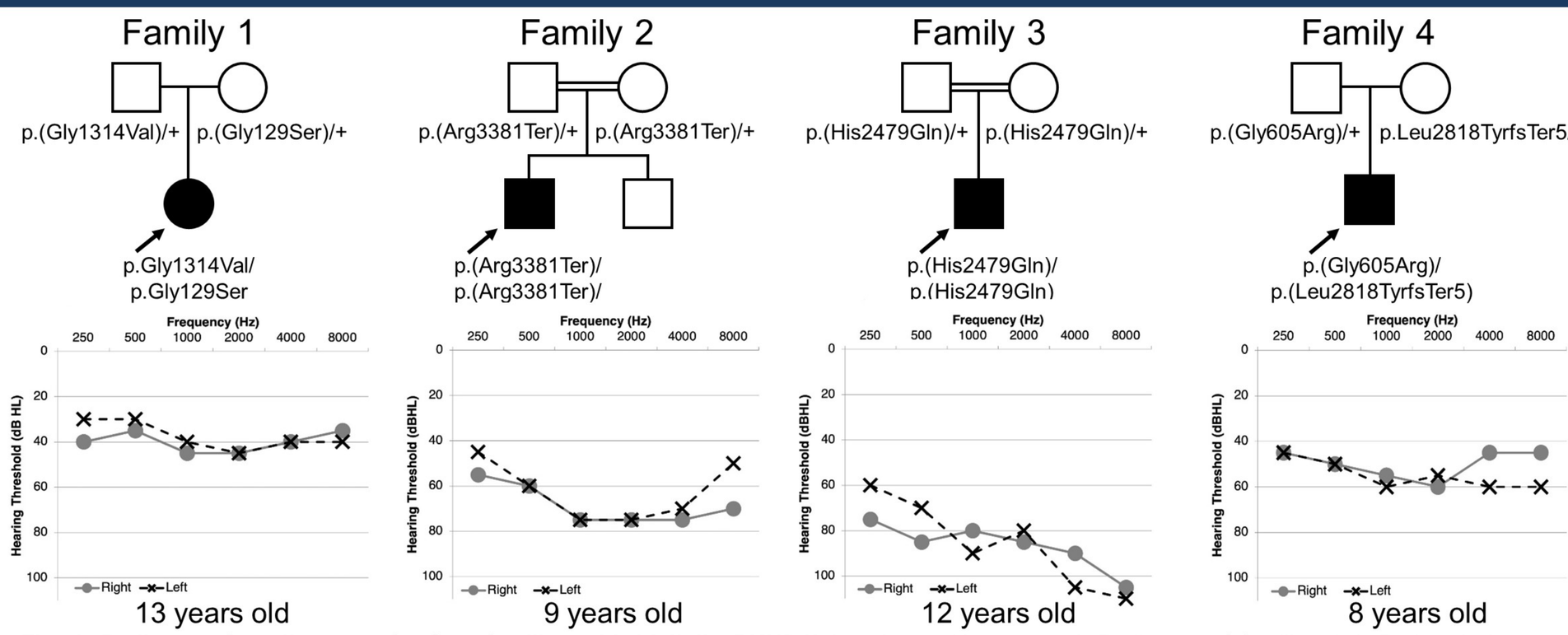

Fig. 1. Pedigrees & audiograms for four families with biallelic *PKHD1L1* variants. Upper panel: Pedigrees of families 1-4. Probands have congenital, progressive, non-syndromic hearing loss without prior family history. Lower panel: Pure tone audiometry for probands 1-4.

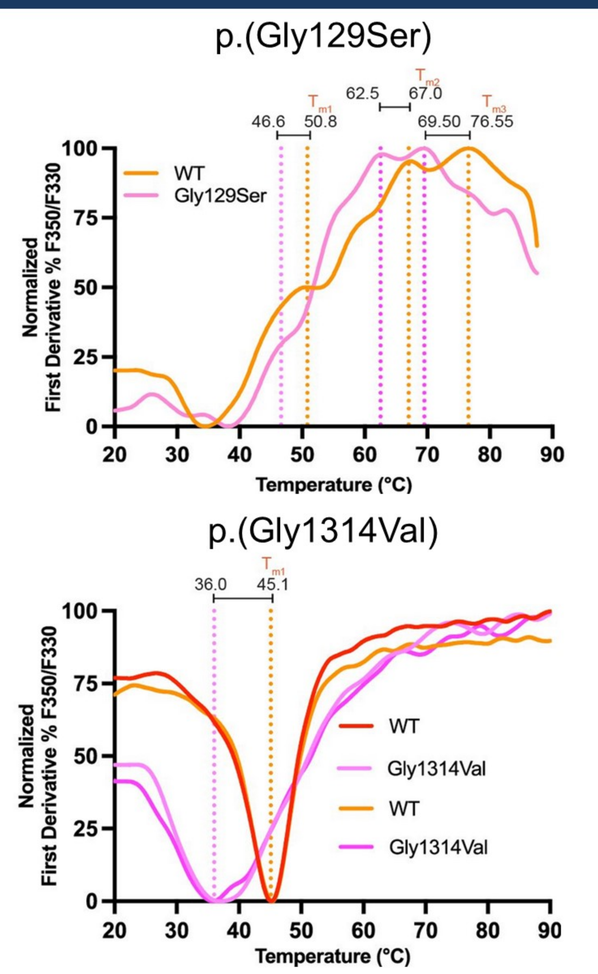

Fig. 2. Thermodynamic and folding stability analysis of p.(Gly129Ser) and p.(Gly1314Val) variants using NanoDSF. Top: NanoDSF melting temperatures for WT IPT1-3 and IPT1-3 p.(Gly129Ser) variant. Measurements show at least three T_m peaks (orange dotted line) for the WT IPT1-3; measured T_m values are shifted left (pink dotted line) showing decreased thermal folding stability. Bottom: Results for WT IPT5-6 and IPT5-6 p.(Gly1314Val) showing reduced thermal stability.

Table 1. *PKHD1L1* variants identified in patients with congenital non-syndromic hearing loss

Family	cDNA (c.) (NM_177531.6)	Protein (p.) (NP_803875.2)	Zygosity	Allele Freq. gnomAD (v.3.1.2)	MAF gnomAD (v3.1.2)	MAF Pop gnomAD (v3.1.2)	SIFT	PP-2	FATHMM	MT	REVEL	CADD
1	385G>A	Gly129Ser	Het	6.6e-6	1.5e-5	European (non-Finnish)	D	D	D	D	N	D
	3941G>T	Gly1314Val	Het	3.7e-4	7.2e-4	Ashkenazi Jewish	D	D	D	D	D	D
2	10141C>T	Arg3381Ter	Hom	2.0e-5	1.9e-4	East Asian	-	-	-	D	-	D
3	7437C>A	His2479Gln	Hom	9.9e-5	3.1e-3	South Asian	D	D	D	B	D	D
4	1813G>A*	Gly605Arg	Het	1.3e-5	1.9e-4	East Asian	D	-	D	D	D	D
	8452_8468del	Leu2818TyrfsTer5	Het	2.6e-5	7.7e-4	East Asian	-	-	-	-	-	D

Abbreviations: MAF, maximum allele frequency; MT, MutationTaster; Pop, population; PP2, PolyPhen-2. Pathogenicity is predicted as D, deleterious; N, neutral; or B, benign; "-" represents not scored.*c.1813G>A is predicted by SpliceAI to cause a donor gain (0.230).

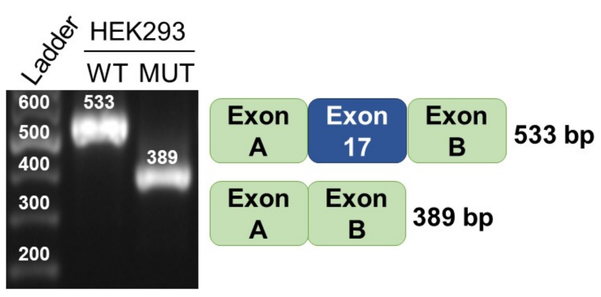

Fig. 3. Minigene assay evaluates splice effects of p.(Gly605Arg). RT-PCR from HEK293 cells transfected with WT or mutant plasmids showed exon 17 skipping, causing an in-frame deletion of 48 amino acid residues. Schematic of calculated fragment sizes with (533 bp) or without (389 bp) exon 17.

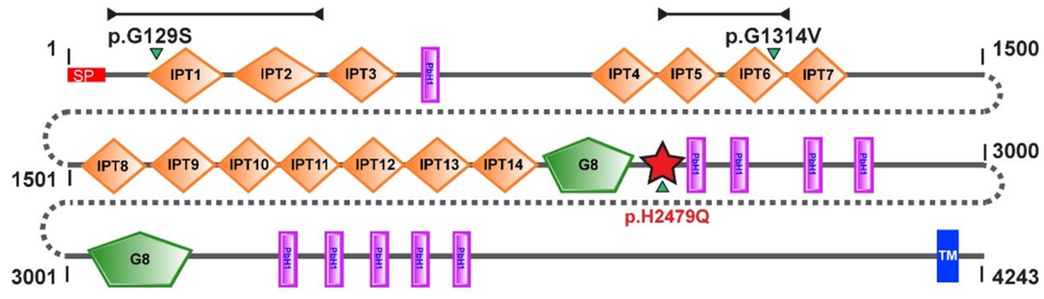

Fig. 4. PKHD1L1 protein domain prediction. Domain prediction from SMART using the *Hs* PKHD1L1 protein sequence. Positions of each missense variant we report are marked with a green arrowhead. The red star represents a newly predicted TMEM2-like domain.

CONCLUSION & DISCUSSION

- We present four families with variable non-syndromic hearing loss, linking *PKHD1L1* to human hearing function as DFNB124.
- Longitudinal clinical and audiological follow-up, expansion of this cohort, and further functional studies will be necessary to strengthen the association of *PKHD1L1* with hearing loss.
- p.(Gly129Ser) and p.(Gly1314Val) substitutions decreased thermal and folding stabilities of recombinant *Mm* PKHD1L1 IPT1-3 and IPT5-6 protein fragments, respectively.
- The c.1813G>A, p.(Gly605Arg) missense variant indicated exon skipping, leading to an in-frame deletion of 48 amino acids (p.Val557_Arg604del).
- New work highlights the role of PKHD1L1 in stereocilia maintenance and susceptibility to permanent hearing loss following moderate acoustic overexposure in PKHD1L1-deficient mice.[3]
- Our recent publication on this gene serves as a call to clinical laboratories to include *PKHD1L1* in hearing loss sequencing panel analysis.[4]

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