Characterization of Hearing-Impairment in Generalized Arterial Calcification of Infancy (GACI)

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Abstract

Background and Importance

Hearing loss (HL) has been sporadically described, but not well characterized, in Generalized Arterial Calcification of Infancy (GACI), a rare disease in which pathological calcification typically presents in infancy.

Objectives

This study aims to describe the clinical audiology and otologic features and potential etiology of hearing impairment in GACI and gain pathophysiological insight from a murine model of GACI.

Design

Cross-sectional cohort study of individuals with GACI. Murine ossicle micromorphology of the *ENPP1^{asj/asj}* mutant compared to wild-type.

Setting

Clinical research hospital; basic science laboratory

Participants

Nineteen individuals with GACI who met clinical, biochemical, and genetic criteria for diagnosis.

Main Outcomes and Measures

Clinical, biochemical, and radiologic features associated with hearing status.

Results

Pure tone thresholds could be established in 15 (n=30 ears) of the 19 patients who underwent audiological assessments. The prevalence of HL was 50% (15/30) of ears, with conductive HL in 80% and sensorineural HL in 20%. Degree of HL was mild to moderate for 87% of the 15 ears with hearing loss. Of those patients with sufficient pure tone and middle ear function data, 80% (8/10) had audiometric configurations suggestive of ossicular chain dysfunction (OCD). Recurrent episodes of otitis media (ROM) requiring pressure-equalizing tube placement were common. In patients who underwent cranial CT, 54.5% (6/11) had auricular calcification. Quantitative backscattered electron imaging (qBEI) of murine ossicles support an OCD component of auditory dysfunction in GACI, suggesting loss of ossicular osteocytes without initiation of bone remodeling.

Conclusions and Relevance

Hearing loss is common in GACI; it is most often conductive, and mild to moderate in severity. The etiology of HL is likely multifactorial, involving dysfunction of the ossicular chain and/or recurrent otitis media. Clinically, this study highlights the importance of early audiology and otologic evaluation in persons with GACI. Novel findings of high rates of OCD and ROM may inform management, and in cases of unclear HL etiology, dedicated temporal bone imaging should be considered.

Background

Generalized Arterial Calcification of Infancy (GACI) is characterized by arterial calcification in early infancy, hypophosphatemic rickets, pseudoxanthoma elasticum, and hearing loss (HL)^{1,2}. Only ~200 cases of GACI have been reported, with an estimated prevalence of 1 in 200,000^{2,3}. Most are due to biallelic variants in the genes encoding ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1) and, less commonly, ATP-binding cassette sub-family C member 6 (ABCC6)⁴. ENPP1 is a transmembrane glycoprotein that cleaves extracellular adenosine triphosphate (ATP) into adenosine monophosphate (AMP) and pyrophosphate (PP_i), the latter of which is a potent inhibitor of mineralization. Although the exact molecule is unknown, ABCC6 is thought to transport ATP into the extracellular space, providing substrate for ENPP1 and being indirectly responsible for PP_i production. Clinically, variable cardiovascular findings predominate in infancy, including widespread arterial calcification, heart failure, respiratory distress, cyanosis, edema, hypertension, and/or cardiomegaly. In survivors, mineral dysregulation and skeletal complications arise, manifesting as hypophosphatemic rickets, peri-articular and entheses calcifications, and cervical spine fusion^{1,2,4,5}. In addition, typical skin and retinal features of pseudoxanthoma elasticum (PXE) and HL have been noted^{1,6,7}.

Reports of hearing status in GACI have been limited in scope; case reports and retrospective reviews have described conductive, sensorineural, and mixed HL diagnosed in infancy or early childhood^{2,7-9}. There has only been one murine study evaluating auditory dysfunction in ENPP1 deficiency, in which the *ENPP1^{asj/asj}* mouse, a model of ENPP1 deficiency, developed progressive conductive HL (CHL), otitis media with effusion, and features of ectopic mineralization of the middle ear structures, including fusion of the malleus and incus and a thickened and overly calcified stapedial artery¹⁰. Given the rarity of disease, auditory function has not been systematically explored in a cohort of patients with GACI. This study aims to describe the extent, severity, complications, and potential etiology of HL in affected individuals.

Methods

Twenty patients with GACI confirmed by biallelic *ENPP1* or *ABCC6* pathogenic variants were referred for audiologic and otologic evaluations at the National Institutes of Health (NIH) Clinical Center, Bethesda, Maryland, USA. All were enrolled in research protocols NCT00369421 and NCT00024804. These studies were conducted in accordance with the Declaration of Helsinki and protocols were approved by the NIH Institutional Review Board (IRB). All subjects or their guardians provided informed consent and assent when appropriate.

Standard audiometric measures included, when possible, air- and bone-conduction pure-tone thresholds for 250 to 8000 Hz and 250 to 4000 Hz, respectively. Clinically significant HL was defined as a 4-frequency (0.5/1/2/4 kHz) pure-tone average (4F-PTA) >20 decibels hearing level (dBHL). Degree of HL was categorized as mild, moderate, severe, or profound¹¹ (Table S1). Type of HL was determined using a 3-frequency (0.5/1/2 kHz) pure-tone average (3F-PTA) and was classified as CHL, sensorineural (SNHL), mixed (MHL), or as having a subclinical conductive component (Table S1). For patients with serial tests, the most recent audiometric evaluation was used for analysis.

Tympanometry and acoustic stapedial reflex thresholds (0.5/1/2 kHz) were conducted to evaluate middle ear function and distortion product otoacoustic emissions (DPOAE) were evaluated to assess cochlear function. Details and interpretation of these procedures are presented in the supplemental materials (Table S1).

Ossicular chain dysfunction (OCD) was defined as: normal hearing and normal tympanogram with elevated or absent acoustic reflexes, or normal tympanogram with air-bone gaps of >10 dB, or presence of a Carhart-notch (e.g. Figure 1). The Carhart-notch is a characteristic artifactual worsening in bone conduction threshold and narrowing of the air-bone gap, typically most pronounced at 2 kHz and suggestive of ossicular chain fixation¹⁵.

Computed tomography (CT) of the head/skull was performed, and in selective cases, temporal bone CT (TBCT) with section cuts of 0.6 mm were evaluated for morphologic abnormalities in the middle ear and otic capsule.

For exome sequencing, DNA was extracted from peripheral blood mononuclear cell. Samples were prepared according to an Agilent SureSelect Target Enrichment Kit preparation guide. The libraries were sequenced with Illumina HiSeq 2000/2500 sequencer. Alignment to the hg19 reference was performed with the Burrows-Wheeler aligner (BWA)¹², deduplication with Picard, and GATK best practice guidelines were followed for variant analysis¹³. Annotation was performed via SnpEff¹⁴.

Since most GACI survivors develop hypophosphatemic rickets and vascular disease over their lifetimes, we evaluated biochemical markers, factors related to mineral homeostasis, and measures of cardiovascular burden of disease. These included intact and C-terminal FGF23 (a phosphate-regulating hormone), blood phosphate, calcitriol and phosphorus supplementation, age of onset of vascular calcifications, the number of ongoing/resolved antihypertensive/heart failure medications, and bisphosphonate use. C-terminal FGF23 was measured at Mayo Clinic Laboratories using human FGF23 C-terminal ELISA kits (Immutopics, Quidel, San Diego, CA, USA). Intact FGF23 levels were measured in-house from plasma using human FGF23 intact ELISA kits (Immutopics, Quidel, San Diego, CA, USA).

Murine model

To characterize mineralization changes that may account for HL, quantitative backscattered electron imaging (qBEI) of the ossicles from *Enpp1*^{asj/asj} mutant, a murine model of *ENPP1* deficiency, and wild-type (WT) mice (C57BL6) was performed using standard procedures as described in the methods section of the supplemental materials.

Statistical Analysis

Descriptive and inferential statistical tests were completed using GraphPad Prism 8 for Windows, version 8.4.1 (GraphPad Software Inc). For comparative analyses, significance for p-values was set at <0.05 and two-tailed, if applicable. Fisher's exact test was used to compare tympanic membrane findings on otoscopy with hearing status. Spearman correlation was used to evaluate intact and C-terminal FGF23, age of calcification onset, age of bisphosphonate (BP) initiation, duration of BP use, age of rickets treatment initiation, and number of hypertensive/heart failure medications—all individually—with the average of air conduction binaural 4F-PTAs.

Results

Prevalence and characteristics of HL

Twenty patients with GACI due to biallelic variants in *ENPP1* were referred for audiology testing; one was excluded from analysis due to cerumen impaction (Tables 1 and 2). Pure tone thresholds were reliably established in 15 patients (10 female, 5 male) with a median age of 8.2 years (range, 4.1-38.5), for a total of 30 evaluable ears. Behavioral audiological evaluations were attempted but unsuccessful in 4 patients due to young age or limited abilities. In these patients, middle ear function and DPOAEs were used to predict peripheral hearing status.

Table 1
Patient characteristics

	GACI patients time of audiology evaluation (n=19)	GACI patients with pure-tone data (n=15)	All patients with hearing loss (n=8)	Patients with CHL (n=7*)	Patients with SNHL (n=2*)
Median age in years (range)	7.9 (0.7-38.5)	8.2 (4.1-38.5)	8.4 (4.1-35.7)	7.9 (4.1-25.5)	22.3 (8.9-35.7)
Age groups (n)					
Children (<10 yrs)	75% (14)	66.7% (10)	75% (6)	83.3% (6*)	50% (1*)
Adolescents (10-19 yrs)	5% (1)	6.7% (1)			
Adults (>=20 yrs)	20% (4)	26.7% (4)	25% (2)	16.7% (1)	50% (1)
Sex					
Female	60% (11)	66.7% (10)	50% (4)	33.3% (3*)	100% (2*)
Male	40% (8)	33.3% (5)	50% (4)	66.7% (4)	
Race/Ethnicity					
Black/African American	10.5% (2)	6.7% (1)			
Multiracial	5.3% (1)	6.7% (1)	12.5% (1)	16.7% (1)	
White	84.2% (16)	86.7% (13)	87.5% (7)	83.3% (5)	100% (2)
*1 patient with asymmetric bilateral hearing loss is counted as both SNHL (right ear) and CHL (left ear); yrs, years; SNHL, sensorineural hearing loss; CHL, conductive hearing loss					

Table 2
Summary of GACI patients with audiology evaluations (n=19)

Age (yrs)	Sex	Right ear					Left ear					Carhart notch	OCD Pattern	History of PET
		Degree of HL	Type of HL	Tymp	AR	Tympanic Membrane	Degree of HL	Type of HL	Tymp	AR	Tympanic Membrane			
0.7	M	.	.	A	.	Normal	.	.	As	.	Normal	.	.	No
2.0	F	.	.	A	.	Normal	.	.	A	.	Normal	.	.	No
4.1	F	Moderate	CHL	A	.	Normal	Moderate	CHL	A	.	Normal	No	Yes	No
4.2	M	.	.	B (PET)	.	PET	.	.	B (perf)	.	Thin; PET Extruded	.	.	PET
4.5	F	Normal	.	A	.	Normal	Normal	.	A	.	Normal	.	No	No
5.1	M	Normal	Normal	C	.	Minimal fluid under TM	Mild	CHL	B (MEE)	.	Yellow MEE	No	No	No
5.8	F	Normal	Normal	A	.	.	Normal	Normal	A	.	Normal	.	No	No
6.3	F	Normal	.	C	Abs	.	Normal	.	C	Abs	Normal	.	No	
7.0	M	Moderate	CHL*	A	.	Thin inferiorly, retracted onto promontory	Severe	CHL*	Ad	.	Monomeric	.	Yes	PET
7.9	F	Mild	CHL*	A	Abs	Normal	Moderate	CHL*	Ad	Abs	Normal	BE	Yes	PET
8.2	M	Normal	SubCl	A	.	Normal	Normal	Normal	A	Abs	Normal	No	Yes	No
8.9	F	Mild	SNHL	C	.	SOM	Mild	CHL	C	.	Retracted, SOM partially filling	RE	Yes	No
9.0	M	.	.	A	.	Normal	.	.	A	.	.	.	No	No
9.7	M	Moderate	CHL*	A	Abs	Normal	Moderate	CHL*	A	Abs	Thin posteriorly	No	Yes	PET
13.1	F	Normal	Normal	A	Elev	Normal	Normal	Normal	A	Abs	Normal	No	No	No
25.5	M	Moderate	CHL	A	Abs	Thickened, no SOM	Mild	CHL*	A	Abs	Thin, retracted	RE	Yes	PET
26.3	F	Normal	.	A	Abs	Normal	Normal	Normal	A	Pres	Normal	No	No	No
35.7 ^a	F	Profound	SNHL*	A	Abs	Normal	Profound	SNHL*	Ad	Abs	Normal	.	.	PET
38.5	F	Normal	Normal	A	Elev	Normal	Normal	Normal	A	Elev	Normal	RE	Yes	No
HL, hearing loss; SNHL, sensorineural hearing loss; CHL, conductive hearing loss; SubCl, subclinical air-bone gaps; Tymp, tympanogram type; PET, past or current pressure equalization tube; AR, acoustic reflexes; Abs, absent; Elev, elevated; TM, tympanic membrane (as observed by otoscopy); SOM, serous otitis media; M, middle ear effusion; RE, right ear; LE, left ear; BE, both ears; NA, not applicable; OCD, ossicular chain dysfunction; PET, pressure-equalizing tubes; NBHS, newborn hearing screening, NR, not reported.														
Periods represent insufficient data. Asterisks and blue-colored row denote hearing aid use in that ear and patient, respectively.														
^a This 35.7 year-old female patient has bilateral EVA and biallelic pathogenic variants in <i>SLC26A4</i> .														
Tympanogram types are defined in Table S1.														

Eight of the fifteen patients who completed pure-tone testing had HL (Table 2); one was unilateral and seven were bilateral, totaling 15 of 30 ears (50%) with HL. The median age of the patients with HL was 8.4 years (4.1-35.7) and included 4/10 female patients (40%) and 4/5 male patients (80%).

For ears with HL, the type was conductive in 80% (12/15 ears) and sensorineural in 20% (3/15 ears). A subclinical conductive component was present in a single ear. There were no patients with MHL. Degree of CHL was mild in 33.3% (4/12), moderate in 58.3% (7/12), and severe in 8.3% (1/12). SNHL was mild in one ear (1/3) and bilaterally profound (2/3) in one patient. This patient with profound SNHL had a diagnosis of enlarged vestibular aqueducts (EVA) and biallelic pathogenic variants in *SLC26A4* (NM_000441.2 c.626G>T (p.Gly209Val), and c.707T>C (p.Leu236Pro)).

DPOAEs were assessed in 19 patients and were present in cases of normal hearing and absent in cases of SNHL or CHL, as expected. For four patients in whom pure-tone data could not be obtained, DPOAEs provided evidence of normal to near-normal hearing sensitivity.

Middle ear function

Tympanometry was performed in 19 patients (38 ears, Table 2). Normal tympanograms (type A) were measured in 68.4% of ears (26/38); of these, eight ears had CHL. Type As and type Ad tympanograms were present in 7.9% (n=3) and 2.6% (n=1) of ears, respectively. Flat (type B) tympanograms were documented in 7.9% (n=3) of ears and were associated with tympanic membrane perforation (n=1), patent pressure-equalizing tubes (PET) (n=1), or middle ear effusion (n=1). Type C tympanograms were recorded in 13.2% (n=5) of ears.

Acoustic reflexes were assessed in 17 of 38 ears. Of these, the reflex was present at normal levels in 5.9% (n=1), absent in 76.5% (n=13), and elevated in 17.7% (n=3). Six of the ears with absent or abnormal reflexes had normal hearing and normal tympanograms. Five ears with absent reflexes occurred in conjunction with CHL and normal tympanograms.

Of 15 patients with pure tone threshold data, 10 had sufficient data for OCD categorization; of these, 80% (n=8) were categorized as having an audiometric pattern consistent with OCD in one or both ears (Table 2); an example of this pattern finding is shown in Figure 1.

Additional audiologic data

Thirteen patients recalled the results of newborn hearing screening (NBHS): of these, nine patients passed and four did not pass (Table 2). The four who failed the NBHS had HL on NIH examination. Three of the six OCD patients with known results passed the NBHS.

Of the 20 GACI patients, five use hearing aids, four bilaterally and one unilaterally. The median age of initial hearing aid fitting was 9 months (6 months-24 years).

Otology findings

Micro-otoscopy revealed abnormal tympanic membranes in 11 of 38 ears examined. Abnormal findings included retracted, thickened, thin/monomeric tympanic membranes and/or presence of serous otitis media (Table 2). Of those with complete audiograms (n=30 ears), abnormal tympanic membranes were seen in eight ears with HL (seven CHL, one SNHL) and one with normal hearing. The remaining 21 ears with audiometric data had normal tympanic membranes on exam; this included seven with HL (five CHL, two SNHL) and one subclinical conductive loss. Excluding the patient with bilateral EVA, abnormal tympanic membrane findings observed on microscopic otoscopy were significantly correlated with hearing status ($p=0.004$, Fisher's exact test; odds ratio, 22.4; 95% CI, 2.4-261.6).

Approximately two-thirds of patients reported a history of recurrent otitis media (ROM) (13/19, 68.4%). Eleven had information regarding the most recent otitis media episode; of these, nine (81.8%) were in early childhood (<6yo), and two (18.2%) were in adulthood (>20 years old). Six individuals (31.6%) had a history of PET placement.

Imaging

Head CT was obtained, often for non-otologic reasons, in 11 patients. However, detailed description of the middle ear, ossicles, otic capsule, and inner ear morphology could not be adequately assessed in most patients due to section thickness (range 1.0-5 mm). In reviewing the imaging, 54.5% of patients (6/11) or 45.5% of ears (10/22) were observed to have auricular calcification (Figure 2G-H). No middle ear effusions were identified.

In two patients with bilateral CHL who underwent TBCT scans, one patient was found to have bilateral auricular calcification, a left-sided abnormal malleus and incus complex (Figure 2A), left-sided high-riding jugular bulb with diverticulum (Figure 2B), and bilateral calcified stapes footplates (Figure 2C-D). The second patient showed bilateral auricular calcification and thickened footplates. Bilateral auricular calcification and bilateral EVA were found in one patient with profound bilateral SNHL (Figure 2G-H).

Relationship between hearing loss and other clinical manifestations

There were no significant correlations (Spearman) between biochemical markers, factors related to mineral homeostasis, and measures of cardiovascular burden of disease with the bilateral 4F-PTA. Details can be found in the supplemental materials.

Quantitative backscattered electron imaging (qBEI) of murine auditory ossicles

Micromorphology, bone mineral density distribution (BMDD), and osteocyte characteristics of murine ossicles were compared by qBEI via unpaired student's t -test, between $ENPP1^{asj/asj}$ and WT mice (Figure 3A). Visual image inspection revealed no major morphologic changes or deformities in the ossicles. In the malleus, no difference was detected in the mean calcium content (Ca_{Mean} , 1.13 ± 1.0 vs. 31.05 ± 0.22 wt%, $p=0.877$; Figure 3B), while $ENPP1^{asj/asj}$ mice showed significantly decreased mineralization heterogeneity (Ca_{Width} , 4.37 ± 0.27 vs. 4.73 ± 1.0 wt%, $p=0.038$; Figure 3C). Analysis of osteocyte characteristics showed a

significantly decreased number of osteocyte lacunae per bone area (N.Ot.Lc/B.Ar, 626.8 ± 132.1 vs. 1163.0 ± 144.6 /mm², $p=0.004$; Figure 3D) and decreased mean osteocyte lacunar area (Lc.Ar, 12.63 ± 0.98 vs. 15.95 ± 0.73 μm^2 , $p=0.004$; Figure 3E) in *ENPP1^{asj/asj}* mice compared to WT mice. In the incus and stapes, the Lc.Ar was similarly higher in *ENPP1^{asj/asj}* mice than in WT (Figure S1E & S2E), but the N.Ot.Lc/B.Ar and BMDD parameters showed no significant differences between groups.

Discussion

This is the first study to systematically characterize hearing impairment in patients with GACI due to ENPP1-deficiency. HL was documented in 53.3% (8/15) of individuals and 50% (15/30) of ears by pure-tone thresholds. CHL occurred in 80% (12/15) and SNHL in 20% (3/15) of HL ears and was mostly mild to moderate in severity. Findings consistent with OCD and recurrent episodes of otitis media were common. In those with head imaging, more than half had auricular calcification.

The predominance of CHL in the present study by standardized, single-institution assessment reflects the literature. Of previously reported cases, 15 have been noted to have hearing impairment (Table S3). Of note, the patients in the present study include 10 previously reported by Ferreira and colleagues²; discrepancies in these two reports including overlapping patients are likely attributed to the rigorous operational definitions of hearing loss in the current study compared to retrospective extraction from clinical notes in the prior.

The specific cause of HL in ENPP1-deficient GACI remains elusive; however, this population was consistently found to have OCD and high rates of ROM. Three of six OCD patients passed the NBHS, suggesting postnatal development of OCD and CHL, possibly due to abnormal ENPP1-mediated ossicle mineralization. Given lacking dedicated imaging and surgical exploration in this cohort, the description of OCD remains broad with the cause of OCD undetermined. Previously, HL in ENPP1-deficient GACI was hypothesized to result from calcification of arteries supplying the middle and inner ear, ossicular chain fixation, and/or an otitis-mediated calcification of middle ear structures (Table S3)^{2,4,7-10}. Thus, we assessed the history and biochemical profile of the present patients. Other factors such as FGF23, hypophosphatemia, age of initiation and duration of rickets treatment, age of initiation and duration of bisphosphonate treatment, number of ongoing and resolved antihypertensive and/or heart failure medications, and age of vascular calcification onset were not correlated with HL.

Micromorphology analyses of murine ossicles support the development of OCD in ENPP1-deficient GACI, suggesting an early loss of ossicular osteocytes without initiation of bone remodeling. In the present study, comparisons between *ENPP1^{asj/asj}* mutant vs. WT murine mallei demonstrated significant decreases in the Ca_{Width} , number of osteocyte lacunae per bone area, and osteocyte lacunae area, with similar trends in the incus. The decreased Ca_{Width} translates to decreased heterogeneity in bone matrix mineralization and contrasts with the expectation of greater variation as typically seen in osteomalacia due to increased osteoid deposition¹⁶. The decreased number of osteocyte lacunae per bone area and lacunae area are consistent with early ossicular aging and have been associated with osteocyte apoptosis in prior studies¹⁷. It is known that ENPP1-deficient mice have fewer and smaller osteocyte lacunae¹⁸. In mice, hypophosphatemia has been shown to regulate perilacunar remodeling, and impairment of perilacunar remodeling is associated with HL^{19,20}. Additionally, HL in GACI was previously studied in the *ENPP1^{asj/asj}* mice, where 90% of mice were observed to have moderate to severe impairment with signs of otitis media and over-calcification of the middle ear, including over-ossification of the round window, fusion of the malleus and incus, tympanosclerosis, and a thickened and over-calcified stapedial artery¹⁰. Taken together, these results suggest an early loss of ossicular osteocytes with loss of perilacunar bone remodeling, causing ossicular chain reconstruction.

Ossicular deterioration in CHL has been demonstrated in other mouse models of FGF23-mediated hypophosphatemia, such as those caused by pathogenic variants in the gene encoding dentin matrix protein-1 (Dmp1), a regulator of phosphate homeostasis and mineralization²¹. However, HL in patients with X-linked hypophosphatemia (XLH), the most common cause of FGF23-mediated hypophosphatemia, is often asymmetric and sensorineural rather than conductive, although some present with MHL.^{22,23} Morphologically, XLH patients have generalized osteosclerosis and significant thickening of the petrous temporal bone with bony remodeling variably observed^{22,23}. Prevalence of HL in XLH ranges from 14-76%²³⁻²⁷. In contrast, there were only two cases of SNHL in our patients with GACI, one of whom had concurrent bilateral EVA.

There is a high prevalence of ROM (56.5%) and increased frequency of PET placement (26.1%) in GACI versus the general population (8.9%), highlighting clinically-significant ROM necessitating intervention²⁸. These findings support previous observations in the *ENPP1^{asj/asj}* mouse, where otitis media with effusion (OME) was observed in conjunction with progressive HL¹⁰. All mutant mice exhibited thickened middle ear epithelium with fibrous polyps, increased mucin-secreting goblet cell, and were found to have OME¹⁰. As shown in our study, ROM may predispose patients to abnormal tympanic membranes, scar tissue in the middle ear space, or ossicular chain fixation/erosion that can lead to chronic HL^{29,30}.

Auricular calcification was observed in more than half of patients with head imaging, the most common CT finding in our cohort and previously observed in murine models³¹. Unlike XLH, there was no demineralization of the otic capsule identified in this study in the few available TBCT scans. The imaging findings of stapes footplate calcification in two patients with CHL suggest that OCD may be responsible for the presence of air-to-bone gaps. The patient with EVA, a common radiologic malformation of the inner ear, had biallelic pathogenic variants in *SLC26A4*, whose gene product, pendrin, transports negatively charged ions across cell membranes^{32,33}. Pathogenic variants in this gene are associated with EVA and early-onset sensorineural hearing loss, as it is hypothesized that pendrin maintains inner ear homeostasis especially during development^{34,35}. EVA itself is associated with some multisystem syndromes, although it is unclear at this time if this finding is coincident and whether a ENPP1-associated pathway plays a role in inner ear development as sensorineural hearing loss possibly from the inner ear vasculature calcification has been reported in GACI⁷. Given the predominance of CHL in this cohort, a second etiology may be possible in the setting of SNHL, as demonstrated in this patient.

Strengths of this study include cohort size given disease rarity and the consistent and comprehensive audiologic and neurotologic evaluations performed at a single institution. Limitations include those inherent to retrospective studies of rare diseases, the cross-sectional design, and inadequate power for certain statistical analyses. It is thus possible that certain analyses for which we found no association could have reached statistical significance with a larger cohort of patients. Future animal studies could establish whether the administration of recombinant enzyme replacement therapy, intended to prevent the cardiovascular and skeletal complications of the disease, prevent, or improve hearing impairment^{29,30}.

Conclusions

Hearing impairment is frequent in patients with ENPP1-deficient GACI, ranging from mild to moderate CHL, and less commonly, SNHL. Novel findings of high rates of suspected OCD and ROM are consistent with a GACI murine model and suggest that patients with ENPP1-deficient GACI are at increased risk for progressive HL, warranting ongoing formal audiologic and otolaryngologic assessments. Early detection of HL may lead to timely treatment, minimizing speech and cognitive sequelae, especially in young patients. While HL from ROM could be treated with PET placement, HL from OCD may be managed with hearing aids and/or assistive listening devices. Select cases may benefit from surgical intervention to restore CHL, including ossicular chain reconstruction and bone conduction implantation. This study highlights the importance of early comprehensive audiologic evaluation with continued monitoring in individuals with GACI.

List Of Common Abbreviations

GACI, Generalized Arterial Calcification of Infancy; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase-1; pyrophosphate (PP_i); HL, hearing loss; CHL, conductive hearing loss; SNHL, sensorineural hearing loss; MHL, mixed hearing loss, 4F-PTA, 4-frequency pure-tone average; 3F-PTA, 3-frequency pure-tone average; DPOAE, distortion product otoacoustic emissions; OCD, ossicular chain dysfunction; PET, pressure-equalizing tympanostomy tubes.

Declarations

Ethics approval, consent to participate, and consent for publication

All patients were enrolled in research protocols NCT00369421 and NCT00024804. These studies were conducted in accordance with the Declaration of Helsinki and protocols were approved by the NIH Institutional Review Board (IRB). All subjects or their guardians provided informed consent and assent when appropriate. Consent for publication is included within the research protocols.

Availability of data and materials

There is no data presented in this study that requires submission to a public repository.

Competing interests

C.R.F. and R.I.G. report a collaboration with Inozyme Pharma, Inc as part of a Cooperative Research and Development Agreement. D.T.B. is an inventor of patents owned by Yale University for therapeutics treating ENPP1 deficiency. D.T.B. is an equity holder and receives research and consulting support from Inozyme Pharma, Inc.

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Author contributions

EHT was responsible for conceptualization, formal analysis, investigation, methodology, writing-original draft, writing-review, and editing. CCB, CKZ, KAK, performed audiological evaluations and were involved in conceptualization, methodology, formal analysis, writing-review, and editing. MMD, TR, MTB were responsible for all aspects of murine investigation, including data curation, formal analysis, methodology, writing, and editing

HJK, RIF, and CRF performed clinical assessments and were involved in conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, validation, visualization, writing-review & editing. All authors read and approved the final manuscript

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Figures

Figure 1

Carhart notch example. Pure-tone audiogram from the right ear of a 25-year-old man with GACI showing a conductive hearing loss with narrowing of the air-bone gap at 2000 Hz characteristic of a Carhart notch. This patient's tympanogram is normal (Type A) and acoustic stapedial reflexes are absent.

Figure 2

Temporal bone CT images of GACI patients. Abnormal findings [A-F] in patient 1, including a coronal view of [A] an abnormal malleus and incus in the left ear (white arrow), a coronal view of a [B] left-sided high-riding jugular bulb with diverticulum (blue arrow), and axial views of [C] right and [D] left calcified stapes footplate (encircled). In patient 2, [E-F], bilateral auricular calcifications (white arrows) and bilateral enlarged vestibular aqueduct were seen (arrowheads).

Figure 3

Micromorphology of murine mallei from *ENPP1^{asj/asj}* mutant mice. Mallei evaluated by qBEI show statistically significant decreases in [C] mineralization heterogeneity (or Ca_{Width} ; $p = 0.038$), [D] number of osteocyte lacunae per bone area (N.Ot.Lc/B.Ar) ($p = 0.004$), and [E] mean osteocyte lacunae area ($p = 0.004$). No change was detected in [B] mean calcium content (or Ca_{Mean} ; $p = 0.877$).

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