Hypothesis: The vestibular aqueduct (VA) in Menière’s disease (MD) exhibits different angular trajectories depending on the presenting endolymphatic sac (ES) pathology, i.e., 1) ES hypoplasia or 2) ES degeneration.

Background: Hypoplasia or degeneration of the ES was consistently found in inner ears affected by MD. The two etiologically distinct ES pathologies presumably represent two disease “endotypes,” which may be associated with different clinical traits (“phenotypes”) of MD. Recognizing these endotypes in the clinical setting requires a diagnostic tool.

Methods: 1) Defining the angular trajectory of the VA (ATVA) in the axial plane. 2) Measuring age-dependent normative data for the ATVA in postmortem temporal bone histology material from normal adults and fetuses. 3) Validating ATVA measurements from normative CT imaging data. 4) Correlating the ATVA with different ES pathologies in histological materials and CT imaging data from MD patients.

Results: 1) The ATVA differed significantly between normal adults and MD cases with ES degeneration, as well as between fetuses and MD cases with ES hypoplasia; 2) a strong correlation between ATVA measurements in histological sections and CT imaging data was found; 3) a correlation between the ATVA, in particular its axial trajectory in the opercular region (angle $\alpha_{exit}$), with degenerative ($\alpha_{exit} < 120^\circ$) and hypoplastic ES pathology ($\alpha_{exit} > 140^\circ$) was demonstrated.

Conclusion: We established the ATVA as a radiographic surrogate marker for ES pathologies. CT-imaging-based determination of the ATVA enables endotyping of MD patients according to ES pathology. Future studies will apply this method to investigate whether ES endotypes distinguish clinically meaningful subgroups of MD patients. Key Words: Computed tomography—Endolymphatic sac—Endotype—Menière—Vestibular aqueduct.

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distinct disease phenotypes exist among patients and whether those phenotypes are caused by different endotypes—i.e., different pathologies affecting the inner ear (2,3). A recent human pathology study (4) was the first to demonstrate two etiologically distinct inner ear pathologies (suspected endotypes) that both affect the endolymphatic sac (ES) (Fig. 1A) and are ubiquitously present among MD patients: 1) degenerative change in the ES (54.2% of cases) and 2) developmental hypoplasia of the ES (37.5% of cases). Correlations with clinical record data suggest that these ES pathologies (endotypes) are associated with different clinical traits (phenotypes). To further investigate the presence of discrete endotype–phenotype patterns based on ES pathologies in patients, clinically applicable methods are necessary to distinguish degenerative versus hypoplastic ES pathology.

Here, we used postmortem temporal bone sections from MD cases and CT imaging data from clinical

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**FIG. 1.** Methods to determine \( \alpha_{\text{entrance}} \) and \( \alpha_{\text{exit}} \), i.e., the angular trajectory of the vestibular aqueduct (ATVA), in histological sections and CT images. A, 3D reconstruction of the endolymphatic space of a human (normal adult) inner ear. The dotted line indicates the opening of the opercular region. B, Endolymphatic duct and endolymphatic sac. Lines \( l_1 \) (red) and \( l_2 \) (green) used for assessing \( \alpha_{\text{exit}} \) are indicated. C and D, Histological assessment of \( \alpha_{\text{exit}} \) in a normal adult temporal bone. E and F, Software-based method to determine \( \alpha_{\text{exit}} \) from temporal bone CT images. See text for details. Scale bars: (D and E) 1 cm. CT indicates computed tomography; LSC, lateral semicircular canal; PSC, posterior semicircular canal; SCC, superior semicircular canal. Images of 3D models for A and B have been adapted from the 3D temporal bone model of the Eaton-Peabody Laboratory Massachusetts Eye and Ear Infirmary, Boston, MA (5).
MD patients to investigate whether different ES pathologies (degeneration versus hypoplasia) are associated with different angular trajectories of the vestibular aqueduct (ATVAs) in the temporal bone. The goal of this study was to establish the ATVA as a radiographic marker to distinguish degenerative from hypoplastic ES pathology in clinical MD patients.

MATERIALS AND METHODS

Ethics
This study was approved by the institutional Review Board of the Massachusetts Eye and Ear Infirmary (IRBNet-ID 880454-1; Boston, MA).

Archival Human Temporal Bone Specimens
From the human pathology collection at the Massachusetts Eye and Ear Infirmary, a total of 136 temporal bone specimens were included from cases with normal age-related audiometric threshold patterns and no history of otologic disease (n = 62), fetuses (abortion samples) with no histological signs of developmental defects (n = 42), and cases with a clinical diagnosis of definite MD (n = 32) (Table 1). All specimens were processed for light microscopy using previously described methods (6).

Temporal Bone CT Imaging Data From Archival Specimens and From Clinical Patients
CT scans of postmortem temporal bone specimens were obtained after tissue removal and fixation and before decalcification, using standard protocols for dedicated high-resolution temporal bone imaging. High-resolution or cone-beam CT imaging of the temporal bones from clinical patients (n = 35; Table 1), all of whom were scanned for suspected diseases not related to the inner ear, was performed without intravenous contrast. All data were reconstructed separately for each temporal bone in the axial plane by using a standard bone algorithm.

Endolymphatic Sac Histopathologies in MD
The histological criteria for degenerative and hypoplastic ES pathology have been described previously (4). Briefly, in degenerative ES pathology, the epithelium in the distal (extraosseous) ES portion exhibits degenerative changes, i.e., pyknotic nuclei, expelled/missing cells, and fibrotic replacement. In hypoplastic ES pathology, the ES is not properly developed and lacks an extraosseous portion.

All specimens with degenerative (n = 18) and hypoplastic ES pathology (n = 14) that were used in the present study have been previously characterized (4).

ATVA Measurements in Histological Sections
The ATVA was determined in horizontally sectioned temporal bone specimens by measuring 1) the entrance trajectory (red arrow, Fig. 1B) of the proximal VA portion into the temporal bone (angle $\alpha_{\text{entrance}}$) and 2) the trajectory along which the VA exits the temporal bone (green arrow, Fig. 1B) in the opercular region (angle $\alpha_{\text{exit}}$). To determine $\alpha_{\text{entrance}}$ (Fig. 1C), we used two points ($P_1$ and $P_2$) on the medial wall of the vestibule, with a distance to the internal orifice of the VA of approximately 1 mm, to define a line $l_0$. A second line $l_1$ was set to run across the orifice, parallel to the most proximal part of the VA. $\alpha_{\text{entrance}}$ is the acute angle enclosed by the lines $l_0$ and $l_1$. $\alpha_{\text{exit}}$ was determined in a different, more caudally located section plane in the opercular region (Fig. 1D). Here, a line $l_2$ was placed parallel to the most distal part of the VA. $\alpha_{\text{exit}}$ is the angle enclosed by lines $l_1$ from Figure 1C and $l_2$, which were merged in a virtual plane (Fig. 1D, inset). $\alpha_{\text{exit}}$ was measured using the angle measurement tool of the software Fiji (7). $\alpha_{\text{exit}}$ was determined using a custom-designed software (see next paragraph and PDF file, Supplemental Digital Content 1, http://links.lww.com/MAO/A755).

ATVA Measurements in CT Images
We modified the method used on histological sections (previous paragraph) for ATVA measurements, since, due to the limits of image resolution, CT did not provide reliable visualization of the VA at its origin from the vestibule. Hence, $\alpha_{\text{entrance}}$ could not be determined in CT images. Instead, a predefined shape (magenta shape, Fig. 1E) was congruently fitted into the bony boundaries of the vestibule and the lateral semicircular canal in the appropriate image plane. The proportions of the shape were determined on an axial CT image from a normal adult temporal bone in which the entire vestibule and lateral semicircular canal were visible. Fitting this shape to more than 100 CT image data sets in this study required only very minor adjustments to the shape’s side ratios, confirming its overall good fit to the normal adult temporal bone anatomy. The line $l_1$ was attached to this shape at a fixed angle of 14.0 degrees, representing the average $\alpha_{\text{exit}}$, as determined from histological sections from normal adult controls (see Results section). Analogous to the method used on histological sections, a line $l_1$ was set to run parallel to the most distal part of the VA in the opercular region in a more caudally located image plane (Fig. 1F). $\alpha_{\text{exit}}$ is the angle between lines $l_1$ and $l_2$ (Fig. 1F, inset).

Software for ATVA Measurements
A custom-made open-source web application was developed for angle measurements from CT imaging data. The software is freely available for download at https://github.com/DanielZuerer/CoolAngleCalcJS or as an online version at https://coolanglecalc.io/.

### Table 1. Groups of cases and patients

| Study Groups                          | Specimens (Cases) | Modality       | Age in Years (Mean ± SD), Except for Fetuses | Sex Distribution |
|---------------------------------------|-------------------|----------------|---------------------------------------------|------------------|
| Normal adults (pathology cases)       | 46 (43)           | Histology      | 55.8 ± 22.3a                                | (Males, Females)  |
| Normal adults (pathology cases) with  | 16 (9)            | Histology and CT| 74.6 ± 17.0a                                |                  |
| available CT scans                    |                   |                |                                              |                  |
| Normal adults (clinical patients)     | 64 (35)           | CT             | 47.7 ± 16.5b                                |                  |
| Fetuses (pathology cases)             | 44 (22)           | Histology      | 76.5 ± 20.1a                                |                  |
| MD, degenerated ES (pathology cases)  | 18 (16)           | Histology      | 82.9 ± 11.2a                                |                  |
| MD, hypoplastic ES (pathology cases)  | 14 (9)            | Histology      |                                              |                  |

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*aAt time of death.
*bAt time of CT scan.
*cP-value of difference between normal adult pathology cases (histology) and normal adult clinical cases (CT).

ES indicates endolymphatic sac; MD, Menière’s disease; n.a., data not available.
Statistical Analysis
Statistical analysis was performed using the software Prism (version 7.0a; GraphPad Software Inc., La Jolla, CA). For group comparisons, one-way analysis of variance was performed, along with post hoc multiple comparison testing by the Holm–Sidak method. For comparison of \( \alpha_{\text{exit}} \) (CT images and histological sections) from adult controls, Student’s unpaired two-sample \( t \) test was used. Pearson’s correlation coefficient (\( r \)) was determined to assess correlations between values for \( \alpha_{\text{exit}} \) as determined from histological sections and CT images from adult controls. For all angles, mean values, and standard deviations are reported. The significance level was set to \( p < 0.05 \).

RESULTS

The ATVA in Temporal Bone Histology: Normal Adults and Fetuses
Normative data for the ATVA (\( \alpha_{\text{entrance}} \) and \( \alpha_{\text{exit}} \)) were determined from histological temporal bone sections. \( \alpha_{\text{entrance}} \) did not differ significantly between normal adults (2D-reconstructed course of a representative VA, Fig. 2A) and fetuses (Fig. 2B) (adults: 14.3 ± 3.8 degrees, range 7.6–22.6 degrees, \( n = 62 \); fetuses: 15.6 ± 6.2 degrees, range 4.5–27.8 degrees, \( n = 42 \); \( p = 0.67 \); Fig. 2E). In contrast, \( \alpha_{\text{exit}} \) was significantly narrower in normal adults than in fetuses (adults: 102.3 ± 9.8 degrees, range, 76.8–119.1 degrees, \( n = 62 \); fetuses: 161.5 ± 14.3 degrees, range, 136.6–188.3 degrees, \( n = 42 \); \( p < 0.0001 \); Fig. 2F). In fetuses, no significant correlation was found between \( \alpha_{\text{exit}} \) and gestational age (\( p = 0.10 \)).

The ATVA in Temporal Bone Histology: Cases With Menière’s Disease
In endotyped MD cases (representative VA morphologies shown in Fig. 2C and D) \( \alpha_{\text{entrance}} \) was determined from histological sections. \( \alpha_{\text{entrance}} \) was not significantly different between the two groups (degenerated ES: 13.8 ± 4.1 degrees, range, 6.0–21.1 degrees, \( n = 18 \); hypoplastic ES: 14.0 ± 3.4 degrees, range, 7.2–19.8 degrees, \( n = 14 \); \( p = 0.97 \)), and neither group significantly differed from normal adults or fetuses (Fig. 2F). In contrast, \( \alpha_{\text{exit}} \) was significantly different between the two MD groups (degenerated ES: 98.9 ± 9.8 degrees, range, 77.5–117.7 degrees, \( n = 18 \); hypoplastic ES: 155.6 ± 11.9 degrees, range, 21.4–220.2 degrees, \( n = 14 \); \( p < 0.05 \)). No significant correlation was found between \( \alpha_{\text{exit}} \) and gestational age (\( p = 0.10 \)).

![FIG. 2. Angular trajectory of the vestibular aqueduct (ATVA, i.e., \( \alpha_{\text{entrance}} \) and \( \alpha_{\text{exit}} \)) measurement in normal adults, fetuses, and Menière’s disease (MD) cases. (A–D) 2D-reconstructed course of the right vestibular aqueduct from multiple (3–6) histological sections in a normal adult case (79 years; A), a fetus (gestational week 8; B), an MD case with a degenerated endolymphatic sac (ES) (94 yr; C), and an MD case with a hypoplastic ES (97 yr; D). (E and F) Values of \( \alpha_{\text{entrance}} \) (E) and \( \alpha_{\text{exit}} \) (F) in normal adults, fetuses, and MD cases (degenerated ES, hypoplastic ES). Insets in (F) illustrate the mean angle (black lines) and the corresponding standard deviations (gray-shaded areas). Statistics: **** \( p < 0.0001 \); CT indicates computed tomography; n.s., not significant. Scale bar: (A–D) 1 mm.](https://danielzuerrer.github.io/CoolAngleCalcJS)
140.9–179.3 degrees, \( n = 14; p < 0.0001 \). Similar values for \( \alpha_{\text{exit}} \) (no statistical significance) were found between MD cases (degenerated ES) and normal adults, as well as between MD cases (hypoplastic ES) and fetuses (Fig. 2F).

**Correlating the ATVA Between Histology and CT: Normal Adults**

To validate ATVA measurements in CT imaging data, we first compared values for \( \alpha_{\text{exit}} \) determined in temporal bone sections from a group of normal adult cases with values determined in CT images from an unrelated group (no significant age or sex differences, Table 1) and found no significant differences (histology: \( 102.3 \pm 9.8 \) degrees, range, 76.8–119.1, \( n = 62 \), data shown in Fig. 2F; CT: \( 99.9 \pm 9.1 \) degrees, range, 80.0–117.3 degrees, \( n = 64 \), \( p = 0.21 \); Fig. 3A). Next, we determined \( \alpha_{\text{exit}} \) from 16 temporal bone specimens that were scanned before histological processing. Values determined from CT images and the corresponding histological sections showed a strong correlation (\( r = 0.78, p = 0.0003 \); Fig. 3B).

**Correlating the ATVA Between Histology and CT: Cases With Menière’s Disease**

To investigate whether the ATVA can be determined in MD cases with degenerative versus hypoplastic ES pathology, we used three postmortem temporal bone specimens from two MD cases that underwent CT imaging before histological processing. Histology demonstrated unilateral degenerative pathology in the first case (Fig. 4A) and bilateral hypoplastic ES pathology in the second case (Fig. 4B). Measurements of \( \alpha_{\text{exit}} \) in both modalities (degenerated ES: Fig. 4C and D; hypoplastic ES: Fig. 4E and F) yielded very similar results with discrepancies of less than 10 degrees (histology – degenerated ES: 87.0 degrees; histology – hypoplastic ES: 165.5 degrees on the left side and 144.6 degrees on the right side; CT – degenerated ES: 88.3 degrees; CT – hypoplastic ES: 160.6 degrees on the left side and 153.6 degrees on the right side; Fig. 4G).

**DISCUSSION**

Numerous previous studies have attempted to identify disease-specific morphological alterations of the VA in MD based on intraoperative anatomy (8), postmortem histology (9–11), CT imaging (12–17), or MRI (18–21). However, those studies did not consider distinct etiologies (endotypes) of MD or their potentially different effects on VA morphology. Moreover, those previous studies that used clinical imaging (CT/MRI) required elaborate postprocessing methods (e.g., 3D reconstructions, (22)) to determine morphological parameters of the VA.

Here, we established the ATVA, in particular the angle \( \alpha_{\text{exit}} \), as a surrogate marker of ES pathologies (histopathological endotypes) in MD (Table 2). By correlating ATVA measurements from histologically processed temporal bones and from CT imaging data, we demonstrated that the ATVA can be reliably determined using clinical imaging data. Our measurements indicated 1) no significant differences in the trajectory of the proximal VA portion (\( \alpha_{\text{entrance}} \)) among all investigated groups, 2) a significant change in the trajectory of the distal VA portion (\( \alpha_{\text{exit}} \)) between the fetal and adult stages of normal temporal bone development, 3) a similar (no significant difference) \( \alpha_{\text{exit}} \) between fetal cases and MD cases with hypoplastic ES pathology, and 4) a similar (no significant difference) \( \alpha_{\text{exit}} \) between normal adult cases and MD cases with degenerative ES pathology.

The observed changes in VA morphology (angle \( \alpha_{\text{exit}} \)) during ontogenesis (fetal versus adult VA; [23], present study) and between MD endotypes (degenerative versus hypoplastic ES pathology; present study) may be attributable to temporal and spatial differences in bone growth.
FIG. 4. Correlation of endolymphatic sac (ES) pathology and $\alpha_{\text{exit}}$ (histology and CT imaging) in Menière’s disease (MD). A and B, Histological sections (opercular region) from a case of degenerative ES pathology (A; inset: degenerated ES epithelium) and a case of hypoplastic ES pathology (B; inset: cyst-like distal end of the ES). (C–F) CT images from the same specimens as in (A) and (B) in the axial focal plane of the opercular region. (E) Correlation of values for the $\alpha_{\text{exit}}$ as determined in CT images and histological sections from the same specimens ($n = 3$, from two MD cases). Dashed line indicates 100% correlation ($r = 1$); scale bars: (A–B) 1 mm, inset in (A–B) 50 $\mu$m, (C–D) 10 mm. Red arrows in (C–F) indicate the opercular region. CT indicates computed tomography.
and development of the petrous bone. The definite size and shape of the bony labyrinth during ontogenesis is thought to be strongly influenced by a decline in the rate of bone metabolism (bone remodeling) in the otic capsule (24), which occurs around the 23rd fetal week (25). At that time point, cells of the membranous labyrinth are presumably starting to secrete osteoprotegerin (OPG), an inhibitor of osteoclast activity, into the perilymphatic fluid spaces, from which the OPG diffuses into the surrounding bone (limited by the diffusion gradient) to inhibit bone matrix remodeling (24). Notably, the distal portion of the VA—which undergoes significant morphological changes between the 19th and 23rd fetal week (23,25, present study)—extends beyond the otic capsule and passes through a layer of normally remodeling (lamellar) bone to the operculum. Prolonged bone growth and continuous bone remodeling in the opercular region during the postnatal period can explain the delayed morphological maturation of the distal VA (compared with the rest of the bony labyrinth), as well as its highly variable morphology in the adult stage (22,26–28).

In the present study, we found that different MD endotypes were associated with significantly different VA morphologies and that the latter resembled very different types associated with significantly different VA stage (22,26–28).

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