



## Hearing loss in fabry disease: A 16 year follow-up study of the Danish nationwide cohort

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### ABSTRACT

**Background:** Fabry disease (FD) is a lysosomal storage disorder resulting in systemic accumulation of globotriaosylceramide (Gb<sub>3</sub>) causing multi-organ dysfunction. The audiologic involvement in FD has been neglected in previous studies; while not a lethal aspect of the disease, hearing loss can have a significantly negative impact on quality of life.

**Objective:** To investigate hearing loss from baseline through 16 years follow-up of the Danish FD cohort and to compare audiometric data to other clinical variables.

**Methods:** Data was collected prospectively and assessed retrospectively during a period of 16 years from 83 patients (age: 9–72 years; sex: 29 males and 54 females). 55 patients underwent treatment. Air conduction thresholds was assessed at six frequencies between 0.25 and 8 kHz bilaterally. Data was analyzed using multi-linear models.

**Results:** Mean follow-up period for patients undergoing a FD specific treatment was 7.8 years (0–12.8 years, SD 3.8 years,  $n = 55$ ). Hearing thresholds for FD patients deviated from healthy individuals at all frequencies for both sexes ( $p < 0.001$ ). Males had more profound hearing loss than females at high frequencies (4.8 kHz) ( $p = 0.025$ ). There was no improvement in hearing with treatment ( $p = 0.343♂$ ,  $p = 0.256♀$ ). No associations between hearing loss and measured glomerular filtration rate, left ventricular wall thickness or cerebral white matter lesions were found. Lower plasma Gb<sub>3</sub> concentration correlated with better hearing ( $p = 0.046$ ) in males.

**Conclusion:** Our findings demonstrated significant hearing loss in FD patients compared to audiologically healthy individuals at all frequencies, and no change in hearing during treatment. Lower plasma Gb<sub>3</sub> concentrations correlated with better hearing in males.

### Synopsis

with Fabry disease despite baseline hearing loss among these patients.

Fabry specific treatment does not improve hearing levels in patients

**Abbreviations:** FD, Fabry Disease; Gb<sub>3</sub>, globotriaosylceramide; mGFR, measured glomerular filtration rate; dB, Decibel; PTA, pure tone average; LVMI, Left ventricular mass index; ERT, Enzyme replacement therapy.

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## 1. Introduction

Anderson-Fabry disease (FD) is an X-linked lysosomal storage disorder that affects glyco- sphingolipid catabolism due to alfa galactosidase deficiency. This results in systemic accumulation of predominantly globotriaosylceramide (Gb<sub>3</sub>) in the plasma and lysosomes, which accounts for a range of clinical complications [1], including but not restricted to angiokeratomas, acroparesthesia, abdominal pain, diarrhea, neuropathy, hypohidrosis, cerebral and ocular affection [1,2]. Furthermore, severe cardiac and renal effects can ultimately lead to cardiac and renal failure [3]. Males are typically more affected than females due to hemizygoty. Conversely, females present as mosaics with varying degrees of symptoms and signs depending on the X chromosome inactivation pattern in the individual [4]. Current specific treatment options consist of intravenous enzyme replacement therapy (ERT) with infusions every second week or oral chaperone treatment.

The audiologic involvement in FD has not been the subject of much research. Although audiologic dysfunction is not lethal, it impacts quality of life, and with increased organ/patient survival of renal (dialysis and renal transplants) and cardiac (implantable cardioverter defibrillator) failure, quality of life becomes more important for survivors. Sakurai and colleagues, demonstrated hearing loss of PTA<sub>4</sub> (= mean hearing threshold in decibels (dB) at 0.5, 1, 2 and 4 kHz) in 9/15 males and 3/12 females with FD when compared to the 90th percentile of a healthy Japanese population [5]. Likewise, in a Portuguese study, 122 patients with FD were found to have worse hearing levels at all frequencies compared to a healthy population [6].

Previously, we presented the hearing status of the nationwide Danish Fabry cohort prior to treatment [7]. In the present study, we investigated the hearing through 16 years of follow-up of the Danish nationwide cohort. Moreover, hearing levels were compared to markers of FD severity such as renal function variables, cardiac ventricular mass, brain magnetic resonance imaging (MRI) and biochemical data. We hypothesized that ERT and chaperone treatment stabilized or, at best, improved the hearing ability.

## 2. Materials and methods

### 2.1. Study design and population

The study was a nationwide longitudinal retrospective analysis of prospectively collected data. All genetically verified Danish Fabry probands and their family members who underwent comprehensive family screening since 2001 were eligible for this study ( $n = 88$ ). Diagnostic criteria for FD included the presence of a FD causing mutation regardless of the alpha-galactosidase A activity in leukocytes. Once a proband was diagnosed with FD, all biological family members were offered genetic testing. If an FD causing mutation was identified, screening for organ manifestations followed. All Danish patients were referred to Copenhagen University Hospital (Rigshospitalet), Department of Endocrinology and Metabolism for single-center diagnosis, treatment plan and follow-up in collaboration with a multidisciplinary Fabry team including many other departments such as Otolaryngology, Nephrology, Neuroradiology and Cardiology.

Only patients with FD and at least one baseline audiogram were included ( $n = 83$ ) (Fig. 1). All audiograms were collected between 2001 and 2017. Patients were either never treated ( $n = 28$ ) or treated ( $n = 55$ ) with ERT (agalsidase-beta or agalsidase-alfa) or an oral chaperone (Migalastat). Seven patients switched to chaperone treatment, however this was towards the end of their follow-up, and due to limited data no attempts have been made to distinguish treatment types. For the never treated patients, the baseline audiogram was defined as the oldest available audiogram. For the treated patients, baseline audiogram was defined as the most recent audiogram available prior to initiation of Fabry specific treatment. Due to limited data in the non-treated group, longitudinal analysis was only performed in the treated group which has in part been described before concerning other organ assessments [7–12].

The study was approved by the Danish Health and Medicine Authority (3–3013-667/1/), the Regional Health Research Ethics Committee (H-3-2014-FSP8) and the Danish Data Protection Agency (2014–641-0055 and 2013-41-1949).

### 2.2. Audiometric definitions and Z-scores

This study incorporated pure tone average (PTA) values, which are

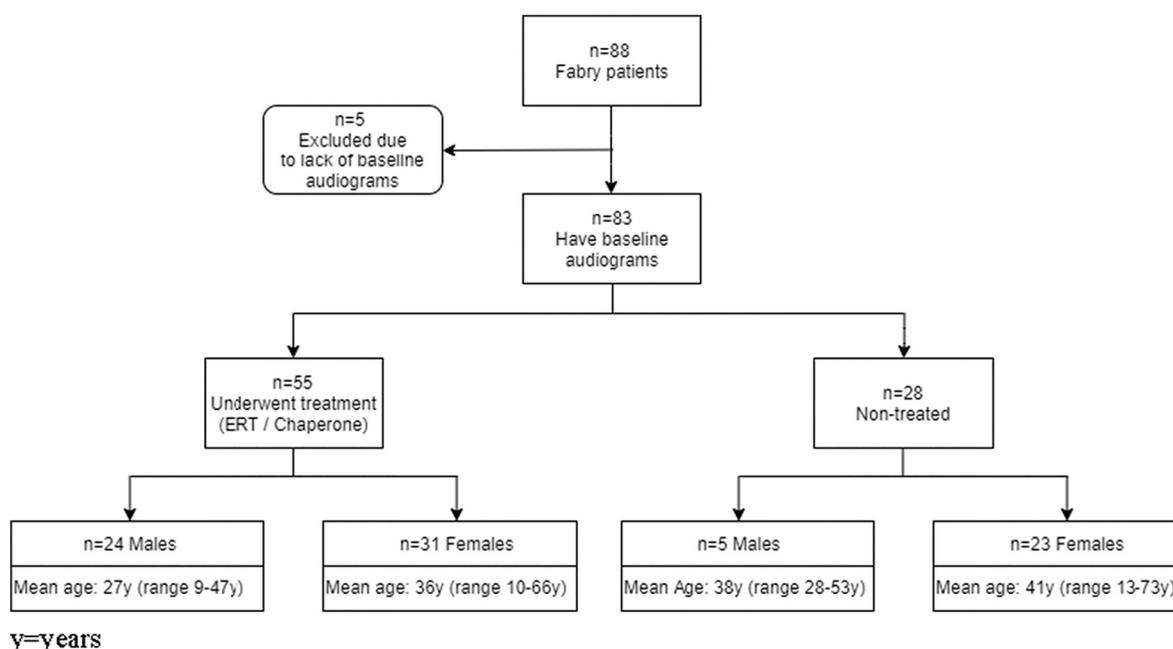


Fig. 1. Characterization of the cohort of patients with Fabry disease.

defined as the mean hearing levels in decibels (dB) of several frequencies ranging from 250 Hz to 8 kHz. PTA<sub>4</sub> was defined as mean hearing levels in dB of 500 Hz, 1 kHz, 2 kHz and 4 kHz. High frequency PTA<sub>48</sub> is defined as mean hearing levels in dB of 4 kHz and 8 kHz. All frequency PTA<sub>6</sub> is likewise defined as the mean hearing levels in dB of all measured frequencies, 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz and 8 kHz and lastly, PTA<sub>3</sub> is defined as the mean hearing levels in dB of 500 Hz, 1 kHz, and 2 kHz. When appropriate, results are standardized as Z-scores (ie. zPTA<sub>4</sub>, zPTA<sub>48</sub>, zPTA<sub>6</sub>), in which sex and age expected hearing loss has been corrected based on data from the International Organization of Standardization ISO 7029 [13]. Z-scores indicate the number of standard deviations the values deviate from the mean of an age and sex matched healthy population (See supplementary material for Z-score calculations, available in supplementary data).

An otologically normal person (i.e. an individual with normal hearing) is defined as a person in a normal state of health who at the time of testing is free from excess wax in the ear canals, is without known ear pathology and who has no history of undue exposure to noise.

### 2.3. Audiometric data

All tests were carried out using pure tone audiometry. Air conduction was in all cases measured in both ears. When the air conduction threshold was poorer than 20 dB hearing level, bone conduction was also performed. Additionally, when appropriate the contralateral ear would be masked. All audiological tests were performed by an audio technician. Tympanometry was not performed on a regular basis. The absence of excess ear wax was ensured through otoscopy prior to all measurements. Pure tone audiometry at frequencies 0.25, 0.5, 1, 2, 4 and 8 kHz was carried out in accordance with ISO 8253-1. The modified Hughson-Westlake technique (-10/+5 dB) was employed using a Madsen Astera Audiometer, Madsen Orbiter OB 922 Clinical Audiometer or Interacoustics AC40 Clinical Audiometer, with Sennheiser HDA 200 circumaural earphones. The equipment was calibrated in accordance with IEC 60318-2, ISO 389-5, and ISO 389-8 using a Brüel and Kjaer 2610 measuring amplifier with a 4144 microphone in a 4152 coupler [14–17].

Reports on hearing impairment were based on WHO's grading of hearing loss [18], which was in turn based on PTA<sub>4</sub> values of the better ear. Moreover, unilateral hearing difference was defined as a hearing difference in PTA<sub>4</sub> of more than 15 dB between the left and right ear. Additionally, speech discrimination scores were determined by presenting each patient with 25 nationally validated Danish words from a Compact Disc. The score was calculated based on the percentage of words a patient could successfully repeat to the audio technician. Each ear was tested individually. In general, a speech discrimination score above 90% was considered normal. All patients could speak Danish.

### 2.4. Other clinical variables

Measurements of glomerular filtration rate (mGFR) values were performed within three months of the auditory assessment. The method used for mGFR was one sample <sup>51</sup>Cr-ethylenedia-minetetraacetic acid (EDTA) clearance technique, previously described in detail [19,20]. All mGFR values have been standardized based on work from Grewal & Blake as Z-scores to correct for the age expected decay in filtration [21,22]. Albuminuria was calculated based on collection of urine through 24 h and analyzed for contents of albumin (see supplementary data for in-depth description) [23].

Cardiac left ventricular mass (LVM) values included were collected within six months of the auditory assessment. All echo cardiograms were completed using a Phillips IE 33 transducer. Measurements of left ventricular thickness and chamber dimensions were performed by two dimensional parasternal images. LVM was calculated based on the American Association of Echocardiography's equation and indexed to body surface area (LVMi) [8].

Gb<sub>3</sub> measurements have been performed at Sahlgrenska institute up to 2006 by densitometric evaluation after high performance thin layer chromatography and orcinol detection [24] and afterwards in Genzyme's laboratories, using a rapid LC/MS/MS method for quantification of total plasma Gb<sub>3</sub> [25]. Given the discrepancy in units between the two laboratories all values have been corrected using the molar mass of Gb<sub>3</sub> (786 g/mol), for coherent statistical analysis (see supplementary table).

Leukoaraiosis and cerebral focal white matter lesions (WML) were quantified both numerically and using Fazekas scale within six months of the most recent audiological follow-up data of all available patents (*n* = 67). Cerebral MRI images were analyzed by a board certified neuroradiologist (VA) using both T2 and FLAIR sequences [26].

### 2.5. Statistical analysis

Data was analyzed using multiple linear modelling with zPTA<sub>4</sub>, zPTA<sub>48</sub> and zPTA<sub>6</sub> as dependent variables with age and other clinical/biochemical measurements as independent variables. Where appropriate a two-sample *t*-test or ANOVA was used. A *p*-value of less than 5% was considered significant (*p* < 0.05). All data was compiled and analyzed in STATA 14.1® (StataCorp, Texas USA).

## 3. Results

### 3.1. Study population

This study comprised 83 patients, 29 males and 54 females. The mean age at baseline was 35 years (yr) (range 9-73 yr, standard deviations (SD) 16.6 yr) for all patients with females being older in average (*p* = 0.012). All but one male and 41 out of 54 females were of the classic phenotype (supplementary table 1 for genotypes). In total 55 (24 males, 31 females) patients underwent treatment and 28 (5 males, 23 females) did not (Fig. 1).

A total of 365 audiograms were included. Mean number of audiometries through the follow-up period per patient was 4.4 for all patients (SD 3.0, range 1–11), and 5.7 for treated patients (SD 2.6, range 1–11). Mean otologic follow-up period among treated patients with follow-up audiograms was 7.8 yr (range 0.9–12.8 yr, SD 3.7 yr) (males 6.9 yr, SD 4.0 yr, range 1.1–12.8 yr); (Females 8.3 yr, SD 3.4 yr, range 0.9–11.7 yr) (males vs females *p* = 0.158). Three male patients under treatment did not have any follow-up audiograms. Only one never treated patients had otologic follow-up data over a period of 12.9 years. All baseline and follow-up data for treated patients is presented in Table 1. Baseline data for both treated and untreated patients are available in a former publication [7].

### 3.2. Individual patient hearing levels and discrimination scores

The following section applies WHO's definition of hearing loss [18]. At any given point from baseline to final follow-up, slight hearing impairment was present in two male patients (6.9%). One female (1.9%) had slight hearing loss and one (1.9%) had moderate hearing impairment. WHO's definition is based on the better hearing ear. When considering the most affected ear, four male patients (13.8%) had slight hearing loss and one male (3.4%) had moderate hearing impairment. A total of five females (9.3%) had slight hearing loss and one (1.9%) had moderate hearing impairment. Two male patients (6.9%) exhibited a unilateral hearing difference at baseline. At follow-up one male (3.4%) and one female (1.9%) still had a unilateral hearing difference.

Mean speech discrimination scores at baseline were 98.7% for males and 99.6% for females with no difference at baseline between treated and never treated patients (*p* = 0.661). At follow-up speech discrimination scores for treated patients were 99.8% and 99.6% for males and females, respectively, and no change from baseline to follow-up of treated males (*p* = 0.423) or females (*p* = 0.083).

**Table 1**  
Baseline and 16 years follow-up data of 55 treated patients with Fabry disease.

	Baseline		Final follow-up visit	
	Females (n = 31)	Males (n = 24)	Females (n = 31)	Males (n = 21)
zPTA <sub>4</sub> (SD)	-0.66 (-2.51-0.20)	-0.97 (-3.2-0.43)	-0.51 (-2.97-0.96)	-0.75 (-3.05-0)
zPTA <sub>48</sub> (SD)	-0.85 (-2.10-0.43)*	-1.56 (-6.34-0.38)*	-0.79 (-2.48-0.28)*	-1.45 (-5.49 to -0.02)*
zPTA <sub>6</sub> (SD)	-0.79 (-2.27-0.22)	-1.09 (-3.36 to -0.11)	-0.56 (-2.22-0.60)	-0.89 (-3.04 to -0.09)
Age (year)	36.4 (10-66.4)*	27.1 (9.4-47.4)*	44.8 (16.4-71.8)*	32.6 (15.0-50.5)*
zmGFR (SD)	-0.46 (-2.73-1.56)	-0.98 (-5.70-1.77)	-0.82 (-3.42-1.15)	-0.96 (-6.12-1.91)
Albuminuria (mg/day)	180 (4-1003)*	811 (4-3787)*	86 (2-390)*	479 (3-2007)*
LVMi (g/m <sup>2</sup> )	108 (58-187)	120 (95-197)	87 (47-188)*	124 (83-306)*
Corrected p Gb <sub>3</sub> (μmol/L)	4.57 (2.27-7.76)*	7.62 (3.56-15.14)*	6.08 (2.93-11.07)	5.48 (2.93-8.65)
A-gal (nmol/h/mg)	15.8 (1.6-39)*	1.9 (0.4-3.1)*	-	-

Baseline and 16 years follow-up data of 55 treated patients with Fabry disease out of a nationwide cohort of 83. First column shows median parenthesis indicates range. SD=Standard Deviation, z = Z-score, mGFR = measured glomerular filtration rate, LVMi = left ventricular mass index, p Gb<sub>3</sub> = Plasma Globotriaosylceramide, A-gal = Alfa-galactosidase activity.

PTA<sub>6</sub> = Pure tone average (mean values of 0.25 kHz 0.5 kHz, 1 kHz, 2 kHz, 4 kHz and 8Khz).

PTA<sub>48</sub> = Pure tone average (mean values of 4 kHz and 8 kHz).

PTA<sub>4</sub> = Pure tone average (mean values of 0.5 kHz, 1 kHz, 2 kHz and 4 kHz).

\* Significant difference between males and females at baseline/final follow-up ( $p < 0.05$ ).

### 3.3. Hearing levels

There was no difference in hearing thresholds between left and right ears of all patients (males  $p = 0.880$ , females  $p = 0.787$ ), thus all data presented will be the mean values of both ears. Additionally, no patients exhibited any significant air bone gap when comparing air-conduction with bone conduction, hence all described hearing loss will be considered sensorineural (cochlear/retro-cochlear).

All patients had the same hearing Z-scores at baseline regardless of them being at different ages at baseline ( $p = 0.277$ ).

Hearing thresholds at all frequencies (zPTA<sub>4</sub>, zPTA<sub>48</sub>, zPTA<sub>6</sub>) deviated from the expected levels of an otologically healthy population ( $p < 0.001$ ). zPTA<sub>48</sub> was different between males and females for both treated ( $p = 0.025$ ) and never treated ( $p = 0.018$ ) patients.

Among treated patients, time in treatment did not change hearing levels for either sex at the tested zPTAs (zPTA<sub>4</sub>, zPTA<sub>48</sub>, zPTA<sub>6</sub>). Hence, hearing levels did neither improve nor worsen in both sexes over the course of treatment compared to the background population. This modelled data is presented in Table 2 and Fig. 2.

### 3.4. Hearing loss, renal function, cardiac mass and biomarkers

In treated patients, mGFR was not associated with zPTA<sub>48</sub> when correcting for age (males  $p = 0.807$ , females  $p = 0.331$ ). Likewise, albuminuria was not associated with zPTA<sub>48</sub> of females undergoing treatment when correcting for age, ( $p = 0.842$ ). However, in treated males, higher albuminuria correlated with better zPTA<sub>48</sub> (beta = 0.00019,  $p = 0.015$ ), zPTA<sub>4</sub> (beta = 0.00017,  $p = 0.019$ ) and zPTA<sub>6</sub> (beta = 0.00019,  $p = 0.014$ ).

There was no relationship between LVMi and zPTA<sub>48</sub> when correcting for age (males  $p = 0.177$ , females  $p = 0.469$ ).

Corrected Gb<sub>3</sub> values could not predict zPTA<sub>48</sub> when adjusting for age in females ( $p = 0.391$ ). However, there was a strong trend of association for increase in corrected plasma Gb<sub>3</sub> values predicting a worse

zPTA<sub>48</sub> in males when adjusting for age (beta = -0.045  $p = 0.065$ ), and there was a significant association to both in zPTA<sub>4</sub> (beta = -0.05,  $p = 0.017$ ) and zPTA<sub>6</sub> (beta = -0.04,  $p = 0.046$ ).

There was no association between the amount of WML and Z-scores for high frequency hearing levels (males:  $p = 0.953$ ; females:  $p = 0.463$ ). Likewise, no association between high frequency hearing levels and the different Fazekas scale groups (males:  $p = 0.561$ ; females:  $p = 0.741$ ).

## 4. Discussion

The present longitudinal analysis of 55 treated patients with FD revealed a presence of hearing loss, compared to the background population, not only before treatment, but also during follow-up, regardless of sex. Hearing loss was more profound at higher frequencies. In addition, males had more profound hearing loss compared to females at all frequencies. No change in hearing levels could be demonstrated over time in treated patients of both sexes in comparison to the background population ( $z = 0$ ). Additionally, increasing Gb<sub>3</sub> levels correlated with worse hearing levels.

Few studies have investigated longitudinal hearing status in patients with FD. A Dutch study described a baseline hearing loss in 16.8% of patients upon using 1980 WHO classification criteria wherein hearing loss was predominantly observed in males (67%) [27]. Their longitudinal analysis revealed that all baseline frequencies deviated significantly from the expected values from healthy individuals, which was in accordance with our findings. In a more recent study by Rodrigues and colleagues, follow-up analysis of 19 patients one-year post ERT, revealed no difference in hearing thresholds in PTA<sub>3</sub> in comparison to baseline values [6]. Likewise, a study by Sergi and colleagues could not demonstrate any difference in hearing levels (PTA<sub>4</sub>, PTA<sub>48</sub>) in 20 patients, 4.5 years after initiating ERT treatment [28]. In contrast, a study from the UK used data from the Fabry Outcome Survey (FOS), to follow 26 patients for an average of 1 year after starting agalsidase-alfa, and observed a significant improvement from baseline to follow-up in most

**Table 2**  
Time in treatment does not improve nor worsen hearing levels.

	Females			Males			M vs F
	P-value	Beta	CI95	P-value	Beta	CI95	P-value
zPTA <sub>4</sub>	0.700	0.003	-0.010-0.015	0.383	0.009	-0.011-0.030	0.238
zPTA <sub>48</sub>	0.579	0.003	-0.008-0.015	0.637	0.008	-0.026-0.042	<b>0.025*</b>
zPTA <sub>6</sub>	0.256	0.007	-0.005-0.019	0.343	0.011	-0.011-0.032	0.068

PTA<sub>4</sub> = Pure tone average (mean values of 0.5 kHz, 1 kHz, 2 kHz and 4 kHz).

PTA<sub>48</sub> = Pure tone average (mean values of 4 kHz and 8 kHz).

PTA<sub>6</sub> = Pure tone average (mean values of 0.25 kHz 0.5 kHz, 1 kHz, 2 kHz, 4 kHz and 8Khz).

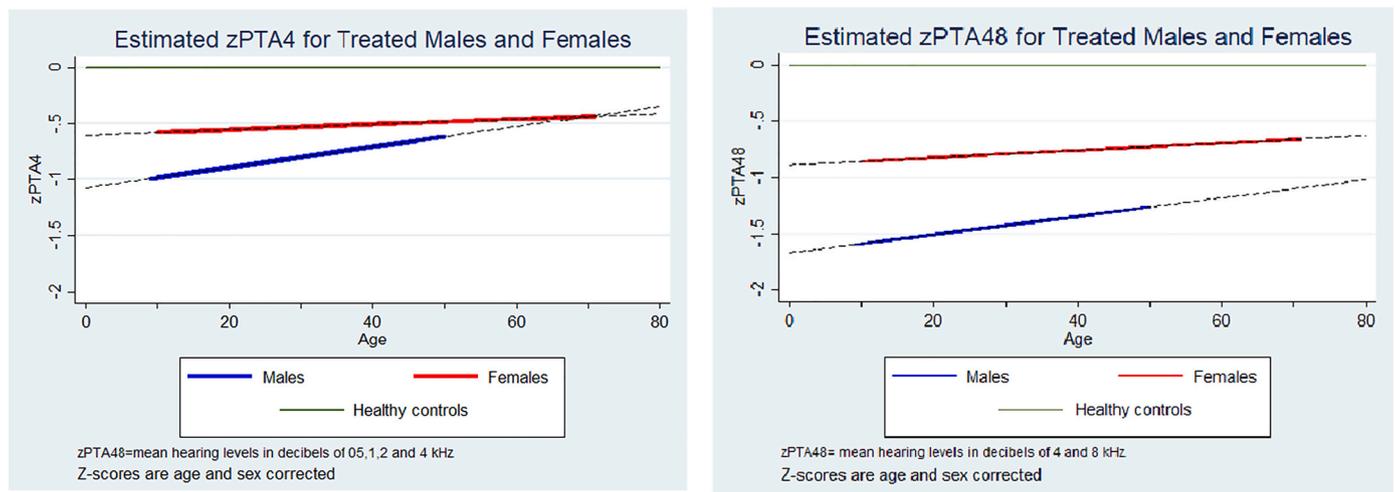


Fig. 2. Development of hearing levels in patients with Fabry disease.

frequencies in patients who initially had moderate degrees of hearing impairment [29]. No improvement was observed in patients with normal hearing or profound impairment [29].

However, there are limitations when only using the FOS registry and agalsidase-alfa treated patients, such as obtaining results from a pre-selected population only. Even more relevant for most above-mentioned studies is the relatively small number of patients with heterogeneous cohort compositions and short follow-up periods.

Our study showed no significant change in hearing over the course of the study, even if the data in Fig. 2 suggests that the hearing slope among male subjects improved over the course of treatment. The lack of significance was potentially due to a small sample size of  $n = 21$ .

The lack of hearing decline over time in patients treated with ERT found in this study, could indicate that ERT stabilizes hearing, or that hearing loss had already occurred early in life before commencement of treatment. The latter explanation was in agreement with a recent study by Suntujs and colleagues investigating the degree of hearing loss in 47 Fabry children [30]. They found that hearing thresholds of children deviated significantly from values of healthy children at all frequencies and most prominently at ultra-high frequencies ( $>8$  kHz). However, this longitudinal follow-up of Fabry patients could not be related to non-treated patients, since only one non-treated patient had audiological follow-up data. All other non-treated patients had only baseline audiological evaluation which we have previously described [7].

We could not establish any correlation between change in hearing levels and mGFR or LVMI in keeping with some studies [5,6]. However these relationships have been observed in cross sectional studies [31,32]. Germain and colleagues observed an association between mGFR and hearing loss in 8/10 men who had mGFR  $<40$  ml/min/1.73m<sup>2</sup> and mean bilateral PTA<sub>3</sub>  $>25$  dB hearing level [31]. We observed an association between an improvement in hearing and higher albuminuria, which contrasts with other findings and logic [6]. This outcome may be a sporadic correlation or an indication that albuminuria can progress in males at an accelerated level despite halted hearing loss by treatment of FD.

No relationship between hearing levels and left ventricular thickness ( $>13$  mm) was observed [30]. Köping and colleagues presented an association between hearing levels (PTA<sub>6</sub>) for both 'Kidney Disease Global Outcome' categories and 'New York Heart Association' class (0 vs 3) in 68 patients [32].

A study from 2007 demonstrated that the load of WML correlated to hearing loss [33], which could not be established in our cohort in keeping with our own baseline data [7]. However, the former study included a majority of male subjects ( $N = 86$ ), while our nationwide cohort based on family screening consisted mostly of female patients. It is possible that a larger sample size of patients or more male patients are

required in order to demonstrate a possible correlation.

A study from 1989 conducted histopathological examination of the temporal bones in two Fabry males, with bilateral hearing loss, sloping towards high frequencies [34]. Atrophy of the stria vascularis and spiral ligaments, with additional loss of outer hair cells was found in the organ of Corti. The authors concluded that both direct and indirect accumulations of Gb<sub>3</sub> resulted in the dysfunction. A weak but significant association between lower Gb<sub>3</sub> levels and better hearing was found in the current study in treated males, which could indicate that reduced Gb<sub>3</sub> concentrations correlated with better hearing overall. Thus, it may be essential to start treatment in early childhood in males with the classical form of Fabry in order to mitigate baseline hearing loss. In females with late onset Fabry, hearing does not seem to be compromised early.

#### 4.1. Strengths and limitations

To our knowledge, we present one of the longest (if not the longest) audiological related follow-up studies of treated patients with FD. Another merit of the study was the inclusion of a nationwide cohort. All Danish patients with FD are followed in a single center, which ensured consistency across all examinations. More importantly all family members were offered genetic testing once a Fabry positive diagnosis was established and complete organ assessment was performed in all patients irrespective of symptoms. Consequently, we have twice the number of females in our cohort compared to males. This allows presentation of a comprehensive unbiased picture of the disease in a national cohort. Presenting sex and age corrected data allowed us to compare function across both sexes and all ages. Nonetheless, the latter can also be considered a limitation as it is highly reliant on the standardization material.

Due to compliance issues, some patients lacked audiometric evaluation and the absence of consistent questioning about vertigo, sudden deafness and self-reported hearing loss limited our otological data. Moreover, we have not performed advanced audiometric evaluation such as auditory brain stem response and vestibular evoked myogenic potential examinations, which could have contributed to further understanding the otologic/cerebral involvement. Given that our Gb<sub>3</sub> results were obtained from two laboratories, it is difficult to accurately compare these results. Hence, it is possible for these assessments to be over- or under-correcting. Also, since a substantial part of our data was collected prior to the lyso-Gb<sub>3</sub> era we could not include this data. Additionally, we have not used a correction for multiple PTA calculations in our statistical analysis. We could also not look at differences in hearing between ERT and Chaperone treated patients due to missing data. Finally, examining 83 patients and smaller subgroups gives a high

risk of type two statistical errors, which is a given limitation when studying rare disorders. This is also more challenging when many patients have mild symptoms and mild organ involvement, which can potentially blur any correlation.

## 5. Conclusion

In conclusion, we observed that our patients with FD had hearing loss compared to the background population from baseline throughout follow-up. Hearing loss was more profound at higher frequencies (4 & 8 kHz) particularly in males. There was no significant improvement in hearing levels over time in treated patients of both sexes. Finally, a lower plasma Gb<sub>3</sub> level in males correlated with better hearing levels. It is possible that ERT and chaperone treatment halts progression of hearing loss in some individuals, but this could not be confirmed.

## Declaration of Competing Interest

U-F-R. has received Advisory Board honoraria from Amicus Therapeutics, Freeline Therapeutics, Sanofi Genzyme, and Takeda, speaker honoraria from Amicus Therapeutics, Sanofi Genzyme, and Takeda, grant support from Shire and Sanofi Genzyme, and is a member of the European Advisory Board of the Fabry and FollowMe Registries.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmgmr.2022.100841>.

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