R705H mutation of MYH9 is associated with MYH9-related disease and not only with non-syndromic deafness DFNA17

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R705H mutation of *MYH9* is associated with *MYH9*-related disease and not only with non-syndromic deafness DFNA17

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Conflict of interest
The authors have no conflict of interest.

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ABSTRACT

MYH9-related disease (*MYH9*-RD) is a rare autosomal dominant disease caused by mutation of *MYH9*, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA). *MYH9*-RD patients have macrothrombocytopenia and granulocyte inclusions (pathognomonic sign of the disease) containing wild type and mutant NMMHC-IIA. During life they might develop sensorineural hearing loss, cataract, glomerulonephritis, and elevation of liver enzymes. One of the *MYH9* mutations, p.R705H, was previously reported to be associated with DFNA17, an autosomal dominant non-syndromic sensorineural hearing loss without any other features associated. We identified the same mutation in two unrelated families, whose four affected individuals had not only hearing impairment but also thrombocytopenia, giant platelets, leukocyte inclusions, as well as mild to moderate elevation of some liver enzymes. Our data suggest that DFNA17 should not be a separate genetic entity but part of the wide phenotypic spectrum of *MYH9*-RD characterized by congenital haematological manifestations and variable penetrance and expressivity of the extra-haematological features.

KEY WORDS

DFNA17 non-syndromic sensorineural hearing loss

MYH9-related disease

Macrothrombocytopenia

p.R705H mutation

INTRODUCTION

MYH9-related disease (*MYH9*-RD) is an autosomal dominant syndromic disorder caused by mutations in *MYH9*, the gene encoding for the heavy chain of non-muscle myosin IIA (NMMHC-IIA) (1, 2). Myosin IIA is a cytoplasmic, non sarcomeric myosin expressed in most cell types and tissues, where it participates in several processes requiring generation of chemomechanical forces by the cytoskeleton (3). As the other conventional myosins, it exists as a hexameric complex containing a dimer of NMMHC-IIA moieties and two pairs of regulatory and essential light chains (4).

MYH9-RD is characterized by a complex clinical phenotype. All the *MYH9*-RD patients have congenital haematological alterations, namely platelet macrocytosis, thrombocytopenia, and characteristic inclusions in the cytoplasm of granulocytes containing both mutant and wild-type NMMHC-IIA. These inclusions are always detectable by immunofluorescence staining for NMMHC-IIA and, when large, they are also visible as basophilic inclusions (also known as Döhle-like bodies) upon conventional staining of blood slides (5-7). The majority of *MYH9*-RD patients develop additional non-congenital extra-haematological manifestations: sensorineural hearing loss (SNHL), presenile cataract, proteinuric nephropathy, and/or a chronic or intermittent elevation of liver enzymes (7, 8). Each of these non-haematological manifestations can occur alone or variably associated with the other ones.

For many years, patients with *MYH9*-RD were diagnosed as having different disorders, such as May-Hegglin anomaly (MHA, OMIM 155100), Sebastian (SBS, OMIM 605249), Epstein (EPTS: OMIM 153650) or Fechtner syndrome (FTNS: OMIM 153640). After the cloning of *MYH9* and identification of mutations in these patients, it was clear that MHA, SBS, EPTS, and FTNS represented different clinical presentations of the same disease (7, 9-11).

At least 70 different mutations causing *MYH9*-RD have been identified so far (12, 13). Only one *MYH9* alteration, p.R705H, was not associated with *MYH9*-RD but with DFNA17 (OMIM 603622), an autosomal-dominant SNHL described in two large unrelated pedigrees (14-15). DFNA17 was reported as a non-syndromic form of SNHL, without any of the other features associated with *MYH9*-RD. Therefore, the current nosography of disorders caused by *MYH9* mutations consists of two entities, the non-syndromic DFNA17 hearing loss due to p.R705H and the syndromic *MYH9*-RD caused by all the other mutations identified in *MYH9*.

We studied four individuals from two unrelated families who also carried the p.R705H substitution. After a comprehensive clinical characterization, they had not only SNHL but also the haematological defects and elevation of liver enzymes typical of MYH9-RD, suggesting that DFNA17 should not be considered a separate entity.

PATIENTS AND METHODS

Patients

All the patients or their legal guardians gave written informed consent for this investigation, which was performed according to the Declaration of Helsinki.

Family 1. The proband (III-1; Fig. 1A) was a male suffering from progressive SNHL since the age of 3 years, who became a candidate for cochlear implantation at the age of 12. Both his 36-year-old mother (II-2) and 62-year-old grandmother (I-2) had received a cochlear implant for progressive SNHL, which was associated with chronic thrombocytopenia. Moreover, individual II-2 had received a clinical diagnosis of FTNS syndrome for the finding of Döhle-like bodies at examination of a bone marrow aspirate.

Family 2. The proband (II-1; Fig. 1A) was a 13-years-old female referred for evaluation of congenital thrombocytopenia. MYH9-RD was suspected for the association of giant platelets with SNHL. There was no family history with clinical features of *MYH9*-RD.

Mutational screening and phenotype studies

Mutational screening of *MYH9* was carried out as previously described (6). The effect of the missense variations was evaluated using four pathogenicity prediction programs, such as PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/) Mutation Assessor (http://mutationassessor.org/), and SIFT (http://sift.jcvi.org).

The methods used for investigation of the patients' phenotypes are summarized in the notes of Table 1 and in Supporting Information.

RESULTS

Identification of the p.R705H mutation

In the probands of both families, mutational screening of the *MYH9* gene identified a heterozygous c.2114G>A mutation in exon 17. The substitution changes an arginine (R) to a histidine (H) at position 705 (p.R705H), a residue located in the head domain of NMMHC-IIA. The same mutation was identified in members I-2 and II-2 of family 1 (Fig. 1A). The parents of the proband of family 2 did not carry c.2114G>A, demonstrating that the substitution occurred as a *de novo* mutation. The amino acid alignment of the 13 human myosin heavy chains of class II showed conservation of arginine 705 in all the proteins (Fig. 1B). Arginine 705 is also conserved among orthologs (Canis lupus familiaris, Bos Taurus, Mus musculus, Rattus norvegicus, Gallus gallus, and Danio rerio; at http://www.ncbi.nlm.nih.gov/homologene; data not shown), suggesting that it exerts a fundamental role in structure and function of the class II myosins. Consistent with conservation data, the bioinformatics tools assigned a pathogenetic score to the mutation (data not shown).

Individuals carrying p.R705H have the congenital haematological features of *MYH9*-RD

The individuals with the p.R705H substitution presented the full typical haematological picture of *MYH9*-RD (Table 1). The immunofluorescence study identified the pathognomonic NMMHC-IIA inclusions in granulocytes in all the four affected individuals but not in their healthy relatives (Fig. 1C). In the three affected subjects of family 1, the inclusions were also evident as basophilic Döhle-like bodies after conventional staining (data not shown). The four patients also had mild thrombocytopenia and marked platelet macrocytosis with giant platelets (Table 1 and Fig. 1C). The degree of platelet macrocytosis was similar to that reported in a cohort of 17 consecutive *MYH9*-RD patients (16).

Non-congenital extra-haematological features of *MYH9*-RD

All the affected individuals had SNHL (Table 1). The patients from family 1 underwent several serial audiometric examinations prior to cochlear implantation, allowing us to characterize the progression of their hearing defect in more detail (Fig. S1; Supporting Information). The self-reported ages at onset of hearing loss were 3, 4, and 19 years for individuals III-1, II-2 and I-2, respectively. Hearing defect was fairly symmetrical, starting in the high frequencies and rapidly deteriorating with age and eventually resulting in profound deafness affecting all frequencies. In individual III-1 the middle and high frequencies were already nearly equally affected at relatively young age. At presentation in our clinic (24 years old), individual I-2 already had severe SNHL (binaural mean AC Pure Tone Average at 0.5, 1, 2, and 4 kHz > 70 dB). The individuals II-2 and III-1 developed severe deafness at the age of 19 and 8 years, respectively. All the affected individuals of family 1 showed significant progression at all frequencies, except for I-2 at 4 and 8 kHz (Fig. S2; Supporting Information); hearing level at 4 kHz was already at 130 dB since the age of 26 years

and hearing level at 8 kHz was already 130 dB since the first audiometric measurement in our clinic at the age of 24 years.

All four affected individuals did not present any signs of kidney damage or cataract (Table 1). Instead, they had mild or moderate elevations of liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) or gamma-glutamyltransferase (GGT), which could not be explained by any concurrent other possible causes of liver damage. The proband of family 2 had ALT 2.2-fold more elevated than the upper normal limit (UNL), whereas AST and GGT were normal. In family 1, individuals I-2 and III-1 had slightly elevated ALT (1.2 and 1.15-fold the UNL, respectively). Individual II-2 only showed elevated GGT of 1.6-fold the UNL. Consistent with the other phenotypic features, the liver enzyme abnormalities were interpreted as part of the *MYH9*-RD syndrome (8, 17).

DISCUSSION

Before the cloning of the disease-causing gene, patients with *MYH9*-RD were diagnosed as having MHA, SBS, EPTS or FTNS, four distinct disorders characterized by associations of the different manifestations of *MYH9*-RD, including macrothrombocytopenia, Döhle-like bodies in granulocytes, SNHL, cataract, and glomerulonephritis (7). MHA and SBS were haematologic diseases with platelet and leukocytes defects. In addition to macrothrombocytopenia, patients with EPTS had the hearing and kidney anomalies (but not Döhle-like bodies and cataract), whereas those with FTNS had the most severe phenotypes with all the features of *MYH9*-RD associated.

However, as patients with *MYH9* mutations were identified, MHA, SBS, EPTS and FTNS lost their distinctive peculiarities (9-11). First of all, finding that Döhle-like bodies were aggregates of NMMHC-IIA allowed the recognition of the granulocyte inclusions in all *MYH9*-RD individuals (18). Independently of their size, they are always detectable using immunofluorescence analysis on peripheral blood smears (5, 7). Moreover, platelet macrocytosis and thrombocytopenia are also present in all the affected individuals, though there are rare exceptions of platelet counts at the lower limit of the normal range (12). Moreover, individuals with *MYH9* mutations, including those previously classified as having MHA or SBS, are at risk of developing SNHL, kidney damage, and/or cataract during life. Finally, studies of large cohorts allowed us to further extend the phenotypic spectrum of *MYH9*-RD finding that more than 50% of affected individuals have elevated liver enzymes (8, 17).

DFNA17 remained the last distinct clinical entity due to *MYH9* mutations. Reported in two families affected by an apparently non-syndromic form of progressive SNHL, DFNA17 was specifically associated with the p.R705H mutation (14,15). To the best of our knowledge, it was not reported whether the affected individuals had defective platelet count and size, or bleeding tendency despite they underwent cochlear implantation.

On the contrary, the patients in this study had thrombocytopenia and large platelets, alterations that associated with SNHL led to a diagnostic suspicion of *MYH9*-RD. The diagnosis was confirmed by finding the NMMHC-IIA aggregates in their granulocytes and identifying the same *MYH9* mutation as that described in the DFNA17 families (14,15). Consistent with the phenotypic spectrum of *MYH9*-RD, our patients also had mild to moderate elevation of some liver enzymes. Of note, the p.R705H mutation was also found to be associated with macrothrombocytopenia, granulocyte inclusions, and SNHL in two patients from the French *MYH9* network (13).

These data indicate that patients with the p.R705H mutation have the syndromic phenotype of *MYH9*-RD. Indeed, the association of macrothrombocytopenia with SNHL, as the only extra-haematological feature, is one of the most frequent presentation of *MYH9*-RD, being reported in 18% of patients at a mean age at evaluation of 35 years (12). Therefore, we conclude that there is no significant evidence to consider DFNA17 as a separate nosological entity from *MYH9*-RD.

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FIGURE LEGEND

Fig. 1. Identification of the p.Arg705His mutation in families 1 and 2. A) Family pedigrees with all the affected individuals carrying a heterozygous c.2114G>A (p.R705H) mutation in exon 17 of *MYH9*; B) Alignment of all human muscle and non muscle myosins of class II with the conserved 705 residue boxed. MYH1 (NM_005963), MYH4 (NM_017533), MYH2 (NM_017534), MYH8 (NM_002472), MYH3 (NM_002470), MYH13 (NM_003802), MYH7 (MN_000257), MYH6 (NM_002471), MYH9 (AB191263), MYH10 (NM_005964), MYH11 (NM_002474), and MYH14 (AY165122). C) Haematological phenotypes in probands of families 1 and 2. In upper panels, immunofluorescence staining for NMMHC-IIA in individuals 1/III-1 (family 1) and 2/II-1 (family 2). Immunofluorescence analysis of a granulocyte from a healthy individual (wt) is also shown for comparison. In the bottom right panel, representative example of platelet macrocytosis of proband 2/II-1 (family 2). At examination of blood smears stained by May-Grünwald-Giemsa, platelets appear exceedingly large, with some platelets even larger than red blood cells. Scale bars correspond to 10 microns.

Table 1. Summary of the results of phenotype investigation of four patients carrying the p.R705H mutations of NMMHC-IIA.

Family/ patient	Age/ gender	Platelet count[1] (x10^9/L)	MPD[2] (µm)	Spontaneous bleeding	NMMHC-IIA leukocyte inclusions[3]	Döhle-like basophilic inclusions[4]	Sensorineural hearing loss[5]	Proteinuria/ kidney failure[6]	Cataract	Liver enzymes elevation[7]
1/III-1	12/M	96	4.0	None	Yes	Yes	Yes	No / No	No	Yes
1/II-2	36/F	115	4.1	Easy bruising	Yes	Yes	Yes	No / No	No	Yes
1/I-2	62/F	142	3.8	Easy bruising	Yes	Yes	Yes	No / No	No	Yes
2/II-1	13/F	107	4.3	None	Yes	No	Yes	No / No	No	Yes

[1]Normal range of platelet count: 150-350 x 10^9/L.

[2]MPD (Mean Platelet Diameter) calculated by software-assisted image analysis on blood slides stained by May-Grünwald-Giemsa (MGG) as previously described (19). The MPD median (25^{th}-75^{th} percentiles) obtained in a series of 17 consecutive *MYH9*-RD patients and in 50 consecutive healthy individuals is 4.2 (3.8-4.5) and 2.4 (2.2-2.6), respectively (16).

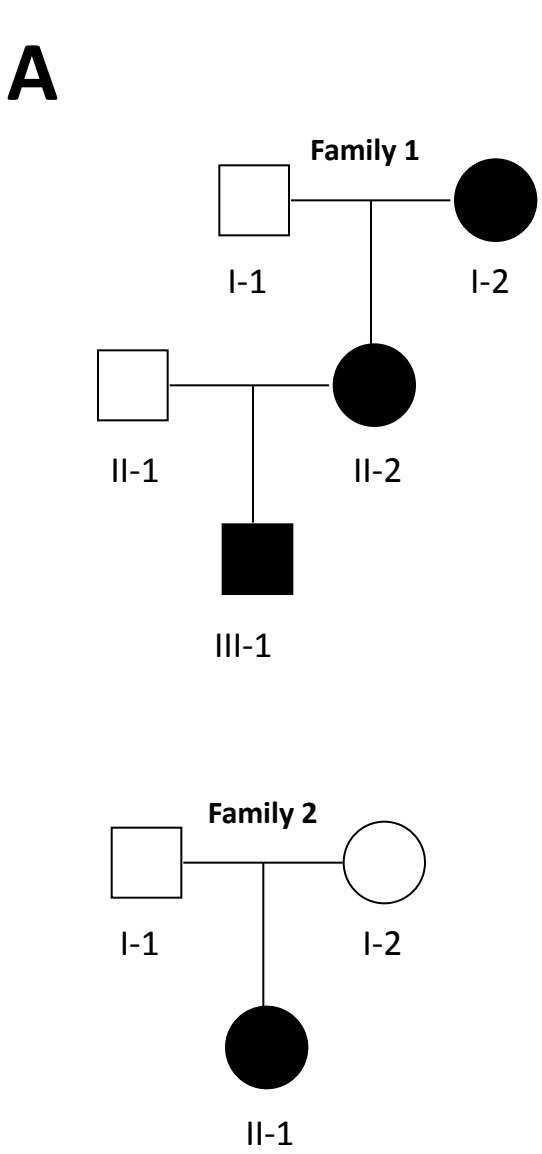
[3]According to immunofluorescence analysis of NMMHC-IIA (non-muscle myosin heavy chain IIA) performed as previously reported (6).

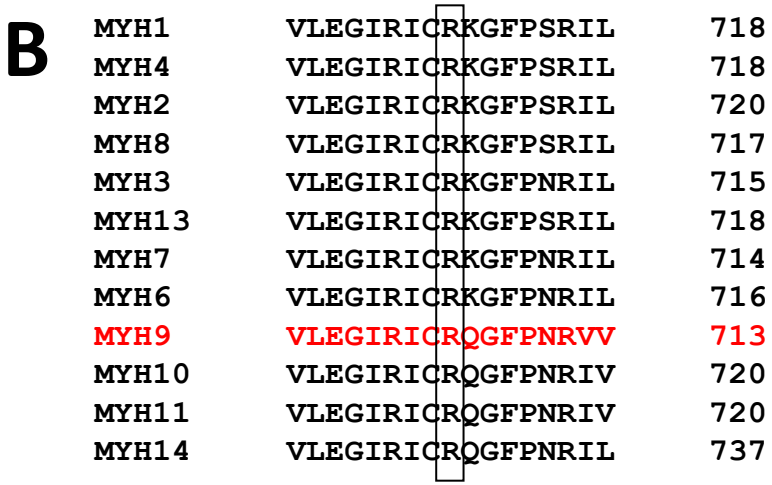
[4]According to examination of blood smears stained by MGG.

[5]Pure tone audiograms were obtained according to standard clinical practice (ISO-389).

[6]Kidney involvement was evaluated by measurement of the protein/creatinine ratio on morning urine samples and by calculation of estimated glomerular filtration rate using the CKD-EPI creatinine equation (20), on at least two different occasions.

[7]Liver involvement was assigned by ratios of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) levels with respect to the upper normal limits (UNL) of the laboratories where the analyses were carried out (8).


A
Family 1
I-1
I-2
II-1
II-2
III-1
Family 2
I-1
I-2
II-1


B
MYH1 VLEGIRICRKGFPSRIL 718
MYH4 VLEGIRICRKGFPSRIL 718
MYH2 VLEGIRICRKGFPSRIL 720
MYH8 VLEGIRICRKGFPSRIL 717
MYH3 VLEGIRICRKGFPNRIL 715
MYH13 VLEGIRICRKGFPSRIL 718
MYH7 VLEGIRICRKGFPNRIL 714
MYH6 VLEGIRICRKGFPNRIL 716
MYH9 VLEGIRICRQGFPNRVV 713
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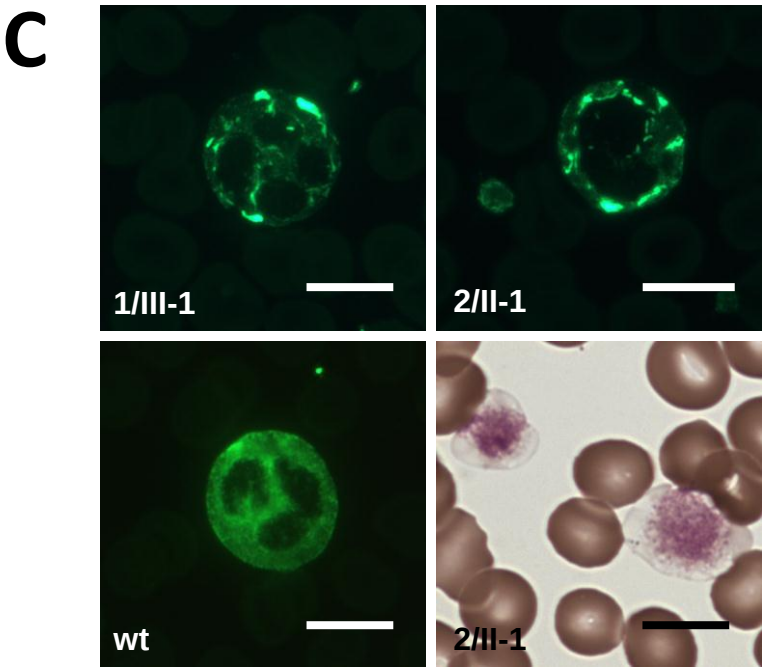
C
1/III-1
2/II-1
wt
2/II-1

Supporting information

R705H mutation of *MYH9* is associated with *MYH9*-related disease and not only with non-syndromic deafness DFNA17

Material and Methods

Hearing evaluation. Pure tone audiograms were obtained according to standard clinical practice (ISO-389). Air conduction (AC) and bone conduction (BC) thresholds were measured at 0.25, 0.5, 1, 2, 4 and 8 kHz. The binaural mean AC threshold at a given frequency was included in the data analysis only if the mean air-bone-gap (ABG) averaged for 0.5-2 kHz was 15 dB or less, and if the thresholds were fairly symmetric. A difference between left and right ear AC of >25 dB for at least 3 consecutive frequencies was labeled as asymmetric. Binaural mean AC thresholds (decibel hearing level) were plotted against age for each frequency for each family member. A commercial program (Graph Pad Prism version 3.0, 1999) was used for linear regression analyses.

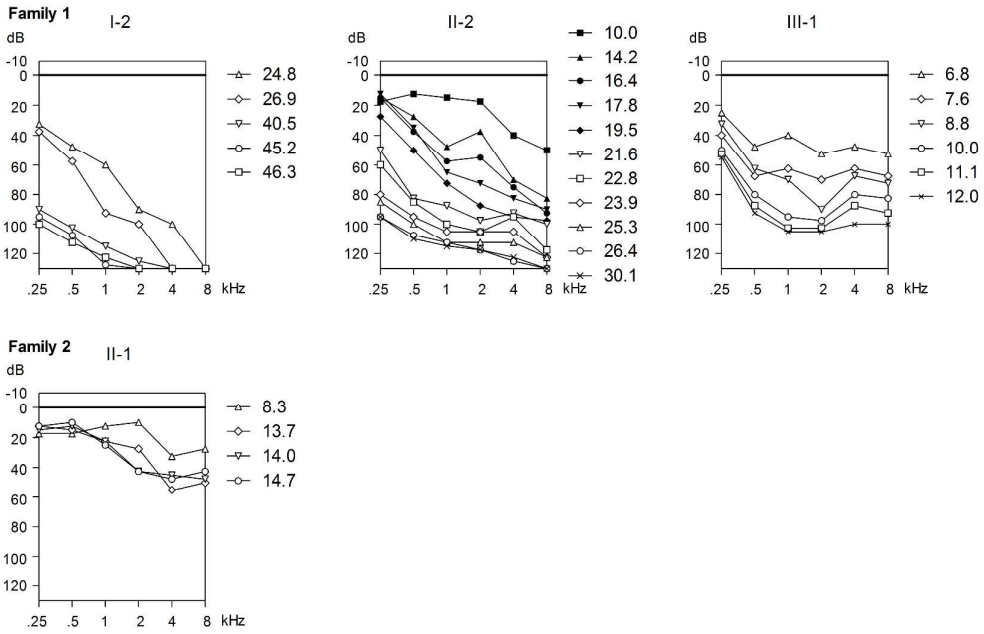

Fig. S1. Longitudinal binaural mean air conduction threshold data of the 4 affected individuals of family 1 and 2. Age in years is shown by symbols next to each graph. For the clarity of the figure not all audiometric measurements are shown.

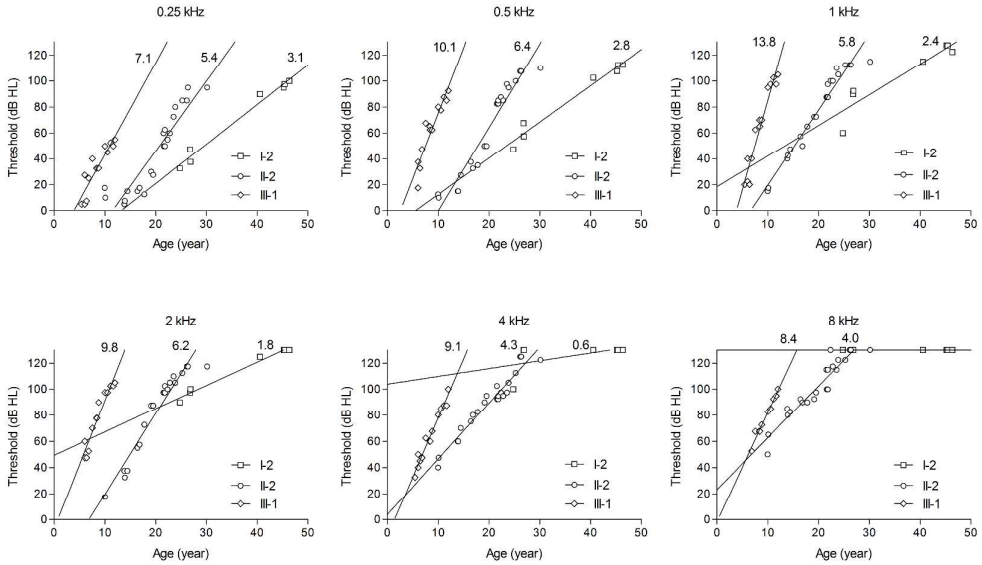

Fig. S2. Longitudinal individual measurement for the 3 affected individuals (I-2, II-2, and III-1) of family 1, with individual linear regression lines (solid lines) are shown for each frequency separately. The annual threshold deterioration (ATD, in decibels per year) is shown above each individual linear regression line.