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A novel missense mutation in *ACTG1* causes dominant deafness in a Norwegian *DFNA20/26* family, but *ACTG1* mutations are not frequent among families with hereditary hearing impairment

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The γ -actin gene (*ACTG1*) encodes a major cytoskeletal protein of the sensory hair cells of the cochlea. Recently, mutations in *ACTG1* were found to cause autosomal dominant, progressive, sensorineural hearing impairment linked to the *DFNA20/26* locus on chromosome 17q25.3 in four American families and in one Dutch family. We report here the linkage of autosomal dominant, progressive, sensorineural hearing impairment in a large Norwegian family to the *DFNA20/26* locus. Sequencing of *ACTG1* identified a novel missense mutation (c.1109T>C; p.V370A) segregating with the hearing loss. Functional analysis in yeast showed that the p.V370A mutation restricts cell growth at elevated temperature or under hyperosmolar stress. Molecular modelling suggested that the p.V370A mutation modestly alters a site for protein–protein interaction in γ -actin and thereby modestly alters γ -actin-based cytoskeletal structures. Nineteen Norwegian and Danish families with autosomal, dominant hearing impairment were analyzed for mutations in *ACTG1* by sequencing, but no disease-associated mutations were identified. Finally, a long-term follow-up of the hearing loss progression associated with the p.V370A mutation in *ACTG1* is provided. The present study expands our understanding of the genotype–phenotype relationship of this deafness gene and provides a sensitive and simple functional assay for missense mutations in this gene, which may assist future molecular diagnosis of autosomal-dominant hearing impairment. Finally, the present results do not indicate that mutations in *ACTG1* are a frequent cause of autosomal-dominant postlingual sensorineural hearing impairment in Norway nor Denmark.

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Introduction

Non-syndromic hearing impairment (NSHI) is the most frequent sensory defect in humans and shows a very high degree of genetic heterogeneity.¹ More than 100 genes are

implicated in this disorder.² At present, 54 chromosomal loci for autosomal-dominant NSHI, 60 loci for autosomal-recessive NSHI and seven loci for X-linked NSHI have been published or reserved.² For these loci, 21 genes associated with autosomal-dominant NSHI, 23 genes associated with autosomal-recessive NSHI and one gene associated with X-linked hearing impairment have been identified.² These so-called deafness genes encode a large variety of proteins, which function in various cell types, structures and processes in the cochlea.^{3,4} The large diversity of deafness genes makes molecular diagnosis of hereditary hearing loss (HL) a difficult task.

Autosomal-dominant HL is estimated to account for 20% of hereditary deafness and, in contrast to autosomal-recessive HL, shows a great variation in age of onset, rate of progression, severity of the hearing impairment and frequencies affected.⁵ It is therefore hoped that in future molecular diagnosis, the particular HL phenotype of a patient can help guide the selection of a limited number of genes for mutational analysis.^{5,6} This approach is already practised in the case of the *WFS1*-associated autosomal-dominant low-tone hearing impairment.⁷ The further success of this approach will be greatly facilitated by the identification and characterization of additional deafness genes/mutations and their associated audiological phenotypes.

Recently, mutations in *ACTG1* were shown to cause autosomal-dominant hearing impairment linked to the *DFNA20/26* locus (OMIM 604717) on chromosome 17q25.3. Five different HL-causing missense mutations in *ACTG1* were described in four American families⁸ and in one Dutch family.⁹ *ACTG1* encodes γ -actin, which is one of six highly conserved actin proteins in human. Actin monomers polymerize to form actin filaments, which are major structural components of the cytoskeleton and which regulate cell shape, cell motility, cell contraction and cell growth. Four actins are muscle specific, whereas β -actin and γ -actin are ubiquitously expressed. Notably, γ -actin is the predominant actin only in the auditory hair cells of the cochlea and in intestinal epithelial cells.^{10,11} The distinct expression pattern of γ -actin compared to other actins is thought to account for the non-syndromic HL phenotype caused by mutations in this gene.⁸

In order to elucidate the genetic basis of dominant HL, we linked autosomal, progressive, sensorineural dominant NSHI in a large Norwegian family to chromosome 17q25.3 and identified a novel missense mutation (p.V370A) in *ACTG1* segregating with the HL. We applied molecular modelling and a cellular assay to investigate whether the mutation may impair γ -actin function and evaluated whether γ -actin mutations are a frequent cause of HL.

Materials and methods

Subjects and DNA isolation

A large Norwegian family (Figure 1a) and probands from five Norwegian and 14 Danish families with autosomal-

dominant hearing impairment (2–5 generations affected, with at least four hearing-impaired family members – details are available upon request) were investigated. None of these families had a specific genetic cause established or were pre-selected based on audiological patterns before inclusion in the study. In addition, eight probands from families with autosomal-recessive or sporadic occurrence of hearing impairment were studied. Genomic DNA was extracted from peripheral blood samples from the HL families as well as from 97 normal hearing individuals (from the same region in Norway as the large family studied) using standard methods. Blood samples were obtained after written consent.

The large Norwegian family was originally described by Teig.¹² We included additional family members and re-examined previously characterized family members; so in total, 40 family members in four generations participated in this study. The follow-up time thereby became 41 years for some hearing-impaired family members. Two of the spouses in the family were hearing impaired: patient VI-2 has profound hearing impairment owing to Rhesus incompatibility, and patient V-1 belongs to a kindred of 11 sibs of whom five were profoundly hearing impaired from birth, although the parents had normal hearing thereby suggesting a recessive inheritance. The fact that his normally hearing parents were first cousins further supports this assumption. Thus, patients VI-2 and V-1 were considered phenocopies and unaffected with respect to *DFNA20/26*. All affected individuals underwent pure-tone audiometry.

Genome-wide linkage scan

Genome-wide linkage analysis in the family described by Teig¹² was performed by amplifying 407 microsatellite markers built on a backbone of the ABI Prism Linkage Mapping Set, version 2 (Applied Biosystems). The average inter-marker distance was 8.66 cM. The PCR products were amplified in 15 μ l reactions containing 50 ng of genomic DNA, 0.33 μ M of each primer, 0.25 μ M of each dNTP, 2.5 mM MgCl₂, 10 mM Tris-HCl, 50 mM KCl and 0.5 U *Taq* polymerase. PCR amplifications were performed in a 9600 or 9700 thermocycler (Applied Biosystems) with the following cycling parameters: 12 min at 95°C; 10 cycles of 94°C for 45 s, 55°C for 1 min and 72°C for 1 min; 20 cycles of 89°C for 1 min, 55°C for 1 min and 72°C for 1 min; 10 min final extension at 72°C. PCR products were pooled based on fluorescent labels and expected allele size range, and electrophoresed through 96-lane 5% denaturing polyacrylamide gels on Applied Biosystems 377 sequencers. The GENESCAN and GENOTYPER software packages (Applied Biosystems) were used for allele sizing and binning. After linkage was demonstrated to 17q25, additional markers (Table 1, online Supplementary material) in this region were genotyped in order to narrow the genetic

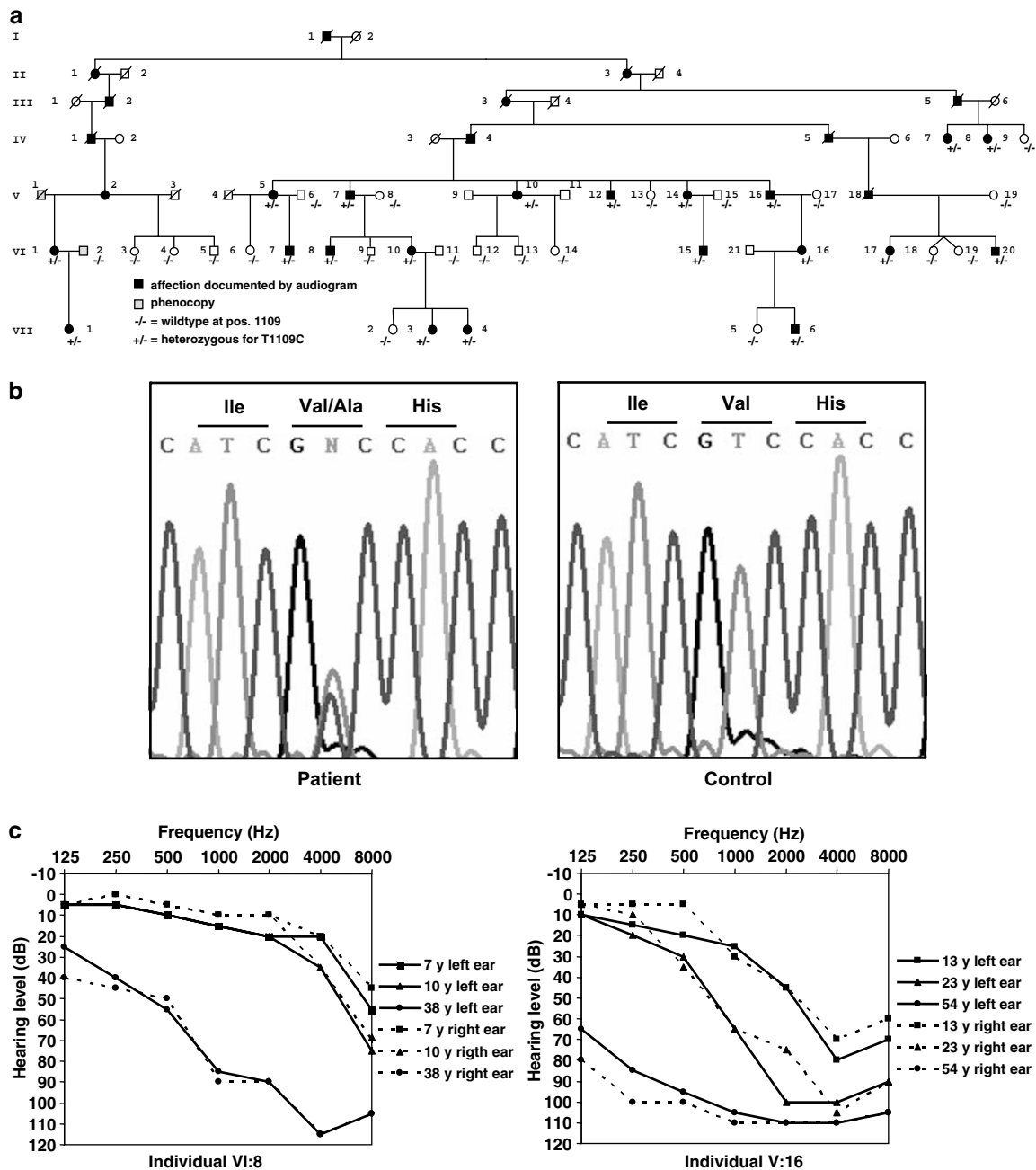


Figure 1 (a) Pedigree of the Norwegian *DFNA20/26* family with autosomal-dominant progressive hearing impairment and segregation of the *ACTG1* c.1109T>C missense mutation. Family members tested positive for the missense mutation are indicated by +/-, and family members tested negative for the mutation are indicated by -/-. (b) γ -Actin mutation analysis. Sequencing result showing the c.1109T>C mutation in *ACTG1* exon 6. The mutation is heterozygous in affected individuals. (c) Audiograms of left and right ear of representative, affected family members (patients VI-8 and V-16). The age at the time of analysis is indicated. The observation period was 31 and 41 years in patients VI-8 and V-16, respectively.

region. Both two-point linkage and multipoint linkage analyses were performed using Vitesse version 1.1.¹³

ACTG1 sequence analysis

PCR for *ACTG1* sequencing in the large Norwegian family was performed on DNA from two affected and two non-

affected family members using primers described by Zhu *et al.*⁸ Following identification of the c.1109T>C mutation, all family members were sequenced in position c.1109. PCR for *ACTG1* sequencing in affected family members from 20 small Norwegian and Danish families segregating autosomal-dominant hearing impairment was

performed by amplifying the coding region (exons 2–6) of *ACTG1* as one PCR product (1840 bp) using the Expand™ Long Template PCR system enzyme mix (Roche Applied Science) and primers 5'-CTTCCCCGCGGGAGGGCAT TAGGT-3' and 5'-GCATGAGGTGTGTGCATTGCGCAGG-3'. Thermocycler conditions were an initial denaturation at 95°C; 40 cycles of 94°C for 20 s, 63°C for 30 s and 68°C for 3 min; with a final extension for 7 min at 68°C. This PCR product was then used as template for exon-by-exon sequencing. Primer sequences are available from the authors upon request. PCR-amplified samples were sequenced by using ³³P and the Thermo Sequenase Cycle Sequencing Kit (Amersham Life Science) or by using Big Dye Terminator™ Cycle Sequencing Ready Reaction Kit according to the manufacturer's instructions.

Analysis of DNA from normal controls for the c.1109C>T mutation

A 258-bp PCR product containing position c.1109 of *ACTG1* was PCR-amplified in 25 µl using the primers 5'-GTGCCTGGCTGTCTTTGCAGA-3' and 5'-CGTGAGGC TAGCATGAGGTGTG-3'. Thermocycler conditions were an initial denaturation at 94°C for 5 min; 40 cycles of 94°C for 20 s, 60°C for 20 s and 72°C for 30 s; with a final extension for 5 min at 72°C. Fifteen microlitres of the PCR sample was digested overnight with 2 U *Hpy8I* (Fermentas) in a 30-µl reaction and analysed on a 2% agarose gel.

Molecular modelling

A model of human γ -actin residues 2–375 was generated using the homology modules in INSIGHT II (2001; Biosym/MSI). The template used for the modelling was the crystal structure coordinates of bovine β -actin crystallized in a complex with human profilin (PDB accession number 2BTE,¹⁴). The program GRID,¹⁵ version 21, Molecular Discovery Ltd, was used to compute hydrophobic surface contours in the model of γ -actin in order to predict residues in γ -actin that engage in hydrophobic interactions with V370. Figures were prepared using the program PyMOL, version 0.97 (<http://www.pymol.org>).

Yeast growth assay

Plasmid pRSWT and yeast strain PR1 were gifts from Dr Peter Rubenstein (University of Iowa, USA). The p.V370A missense change was introduced into the actin gene in plasmid pRSWT by site-directed mutagenesis. After transformation of the wild-type or mutant plasmid into yeast (PR1) and selection with 5'-fluoroorotic acid, the yeast cultures were grown in TRP selection medium at 30°C overnight, and then grown in YPD medium for 6 h with starting OD₆₀₀ = 0.1. The cells were then plated on agar plates with YPD or YPD + 0.5 M NaCl at 5, 5 × 10, 5 × 10² or 5 × 10³ cells/spot. The cells were incubated at 30 and 37°C for 3 days and then analysed.

Results

Linkage to the *DFNA20/26* locus

Before the genome-wide screen in the family described by Teig,¹² we investigated possible linkage to various known NSHI loci (*DFNA2-DFNA7*, *DFNA10*, *DFNB2*, *DFNB6*, *DFNB8* and *DFNB10*), but linkage to any of these loci could be excluded (data not shown). We also sequenced *GJB2* in seven affected (VI:20, VI:17, V:16, VI:10, VI:2, VI:1 and IV:7) and two (VI:18 and V:19) normal hearing family members because of its prevailing aetiological role in hereditary, including dominantly inherited deafness (<http://davinci.crg.es/deafness/>). Individuals VI-17 (affected) and V-19 (unaffected) were found to be heterozygous for the p.M34T missense change. No other *GJB2* mutations or polymorphisms were identified. The lack of co-segregation between the p.M34T allele and the hearing impairment indicates that this mutation does not play a major role in HL in the family.

A genome-wide scan identified a putative locus on chromosomes 16 and 17. The region on chromosome 16 between markers D16S3075 and D16S415 (maximum LOD score of 1.7) was analysed with additional markers (D16S519, D16S3041, D16S420, D16S690, D16S3396 and D16S771) but negative LOD scores were obtained for these markers. In contrast, genotyping of additional markers (D17S1807, D17S802 and D17S668) at chromosome 17q25 strengthened linkage to this region with a maximum two-point LOD score of 5.47 (Theta = 0) at marker D17S668. Multipoint linkage analysis gave a strong peak (multipoint LOD score of 9.16) between markers D17S784 and D17S928. By examination of haplotypes using genotypes of five markers, the minimum candidate region, limited by recombination events in hearing-impaired individuals, is flanked by markers D17S784 and D17S928 representing a distance of 6.1 cM on the deCode genetic map. Linkage to this chromosomal region has previously been reported for autosomal-dominant HL and two overlapping hearing impairment locus numbers, *DFNA20* and *DFNA26*, have been assigned to the region.^{16,17} Recently, *ACTG1* was found to be the causative gene for hearing impairment in this region in five American and Dutch families (Table 2, online Supplementary material).^{8,9}

Mutation analysis of *ACTG1*

In order to determine whether *ACTG1* is mutated in the family described by Teig,¹² sequencing of the *ACTG1* coding region (GenBank no. X04098.1 (mRNA sequence) and GenBank no. M19283 (genomic sequence)) in two affected and two unaffected members of this family was performed. The analysis identified a potentially disease-causing c.1109T>C nucleotide transition in exon 6 of the affected family members, which results in substitution of alanine for valine in position 370 of γ -actin (Figure 1b). DNA from 19 affected and 21 non-affected family members was thereafter analysed and the mutation showed perfect

co-segregation with the HL in all 19 affected individuals. Furthermore, 97 normally hearing individuals originating from the same geographic location in Norway (Trøndelag, Mid-Norway) as the present family were analyzed for the mutation. This analysis was performed by *Hpy8I* digestion of PCR-amplified genomic DNA, as the T-to-C transition of the mutation destroys a cleavage site for this endonuclease. The mutation was not found in any of the normally hearing individuals. V370 is conserved in all human actin isoforms as well as in actins from mouse, fruitfly, worm, yeast and slime mold. The evolutionary conservation of V370, the co-segregation of the p.V370A mutation with HL and the absence of the mutation in normal hearing control individuals strongly indicated that this mutation underlies the HL observed in the Norwegian family.

The p.V370A mutation impairs growth of yeast cells

In order to test directly whether the p.V370A mutation impairs actin function, we performed growth assays with yeast (*Saccharomyces cerevisiae*) expressing wild-type yeast actin or yeast actin with the presumed deafness mutation. Yeast expresses only one actin, which is 89% identical to human γ -actin and in which the valine is conserved at amino-acid position 370. The p.V370A mutation was found to affect growth of yeast only slightly under normal growth conditions, that is, growth on YPD agar plates at 30°C (Figure 2a). By contrast, the p.V370A mutation severely impaired growth during hyperosmolar stress (Figure 2b) as well as growth at elevated temperature

(Figure 2c). When both stresses were applied together, the p.V370A mutation completely suppressed growth (Figure 2).

Structural consequences of the p.V370A mutation

We next investigated the structural consequences of the p.V370A mutation. Owing to a high degree of sequence conservation (99% identity), the crystal structure coordinates of bovine β -actin, complexed with the actin-regulatory protein profilin, could be used to generate a reliable model of human γ -actin using the Inside homology modelling program (Figure 3). Analysis of the model using the program GRID,¹⁵ showed that the side chain of V370 engages in hydrophobic interactions with the side chains of Arg116, Val134, His371 and Cys374 (Figure 3b) and these interactions appear to be a major determinant in stabilizing the position of the C-terminus of γ -actin (residues 368–375). Superimposition of several actin structures from various species^{18–21} indicated that V370 has a conserved role in stabilizing the position of the C-terminus of actins via these hydrophobic interactions (data not shown). Importantly, analysis of a model of the p.V370A mutant revealed that the side chain of A370 is too short to establish hydrophobic interactions with Arg116, Val134, His371 and Cys374 (Figure 3c). Otherwise, the p.V370A mutation is not predicted to alter the structure of γ -actin. Thus, molecular modelling analysis strongly suggested that the p.V370A mutation affects γ -actin function by destabilizing the position of the C-terminal tail.

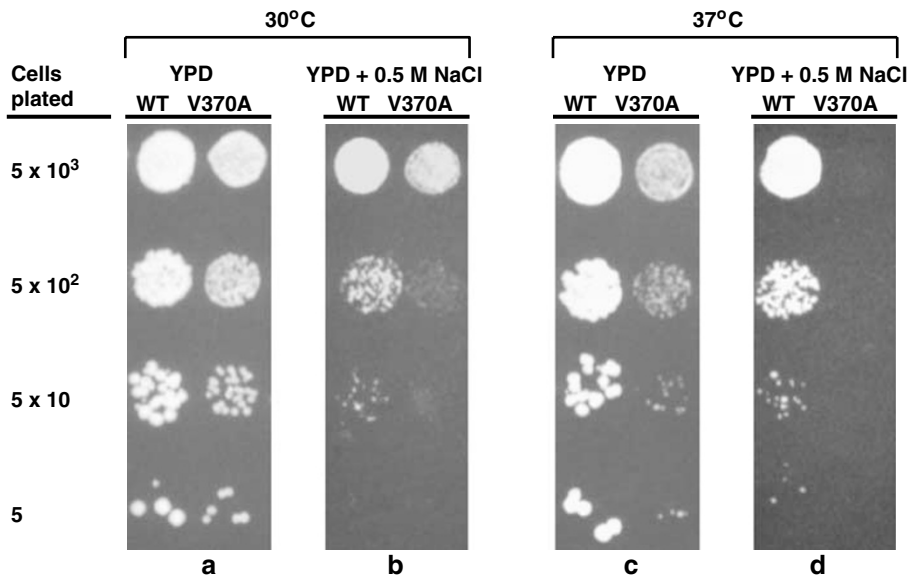


Figure 2 Yeast growth assay. Yeast cells expressing either wild-type or p.V370A mutant γ -actin were plated on YPD or YPD + 0.5 M NaCl agar plates at various densities and incubated at 30 or 37°C. The photograph shows the cultures 3 days after plating. The experiments were repeated three times with similar results.

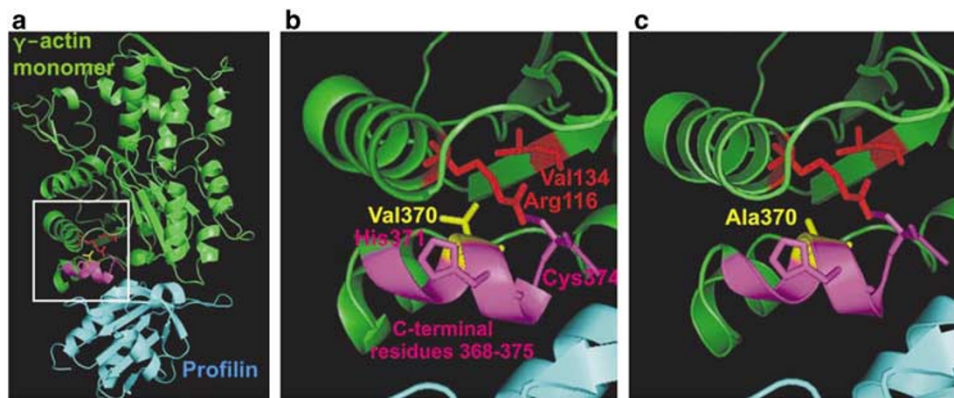


Figure 3 Modelling of γ -actin. (a) Structural model of wild-type γ -actin (green) in complex with profilin (blue) shown as ribbon representations with side chains of residues discussed in the text. V370 is shown in yellow, and the C-terminus of γ -actin and residues herein that interact with V370 in magenta. Residues elsewhere in γ -actin that interact with V370 are shown in red. (b) Enlarged view of the boxed region in (a). (c) As in (b), except that γ -actin is the p.V370A mutant.

Phenotype of HL associated with the p.V370A mutation

The present family shows non-syndromic, autosomal-dominant hearing impairment with complete penetrance and variable onset. The hearing-impaired individuals present bilateral, sloping, progressive sensorineural hearing impairment. Based on 19 persons belonging to the three youngest generations, the average age for diagnosis of hearing impairment was 35.3 years. However, the average age of onset of hearing impairment is surely much lower, as, particularly older, members of this family living in a remote valley in mid-Norway tended to seek medical help for diagnosis of hearing impairment fairly late. Considering mainly the younger members of the family, the age of diagnosing hearing impairment ranged from age 6 (in VII:6) to age 24 years (in VII:1). We estimate that the average age of onset of hearing impairment is in the first or second decade. In agreement with this estimate, most of the affected members of the family needed hearing aid from about age 20 years. As described by Teig,¹² the HL first affects the highest frequencies, frequently around 7 years of age, and progresses to 60–80 dB HL for frequencies >1000 Hz. It then progresses to a 40–60 dB HL for frequencies <1000 Hz. Further progression leads to profound hearing impairment for frequencies >1000 Hz in the majority of the affected individuals, gradually presenting as a flat or corner audiogram. Our subsequent audiological examination of the family is in very good agreement with the findings by Teig.¹² Figure 1c shows two representative audiograms from the family, which illustrate the progression of the HL over 31 and 41 years, respectively, thereby providing a long-term follow-up time of examination. In Supplementary Figure 1 is shown an audiogram of the earliest documented age of onset of hearing impairment in the family, which was at 6 years of age. As reported by Teig,¹² the development of HL generally followed a similar pattern in affected individuals. In the present study,

however, we noted examples of significant variation in the rate of progression of HL, as illustrated in Supplementary Figure 1, showing audiograms from two female subjects recorded at similar ages. We were not able to determine whether gender affected the HL phenotype.

Three affected family members (patients V-12, V-16 and VI-15) received a cochlear implant at the ages of 63, 58 and 43 years, respectively, but had limited benefit from the device. However, they also did not attend audiological speech rehabilitation and kept being primarily sign language users despite being able to sense sound after the implantation. Our observations were too anecdotal to determine if their limited benefit of cochlear implant was due to lack of compliance with speech rehabilitation or indicated a degenerative component affecting central auditory pathways.

As described above, individual VI:17 had a p.M34T *GJB2* mutation in addition to the *ACTG1* mutation, raising the question whether this individual might display a more severe HL due to the double mutation. However, individual VI:17 had at age 36 years a PTA_{0.25–8 kHz} (pure-tone average audiogram over the frequencies 0.25–8 kHz) of 41/36 dB HL for left/right ear, whereas her hearing-impaired brother (VI:20), also with the *ACTG1* mutation but with normal *GJB2* sequence, had a PTA_{0.25–8 kHz} of 58/62 dB HL left/right ear at age 31 years. To further investigate the p.M34T *GJB2* mutation, we performed protein expression analysis of connexin 26 (the protein encoded by *GJB2*) and 43 in cultured skin keratinocytes from a skin biopsy of individual VI:17 as well as of VI:18 and V:19 (with normal *GJB2* sequence), as described.²² This showed immunohistochemical signals of similar intensity for both connexin 26 and 43 in keratinocytes from all three individuals (data not shown). Taken together, these results indicate that the p.M34T *GJB2* mutation is not likely to affect the deafness phenotype of the p.V370A *ACTG1* mutation.

Vestibular dysfunction, manifested as some equilibratic instability, was claimed by some of the elderly, profoundly hearing-impaired individuals, but was not formally tested in the family.

Mutation analysis of *ACTG1* in families with unknown cause of autosomal-dominant hearing impairment

Finally, we wished to elucidate whether mutations in *ACTG1* may be a frequent cause of autosomal-dominant hearing impairment. The entire coding region and intronic splice junctions of *ACTG1* were therefore sequenced in affected members from 19 small Norwegian and Danish families with dominantly inherited hearing impairment of unknown cause. In addition, eight probands from families with autosomal-recessive or sporadic occurrence of hearing impairment were analysed. In this analysis, we included families with autosomal-recessive HL, because certain deafness genes (*TMCI1*, *TECTA*, *MYO7A*) have been found to underlie autosomal-dominant as well as recessive HL, and we therefore wished to investigate whether that might also be the case with *ACTG1*.

Six nucleotide changes were found in *ACTG1* in the 27 families, but none of the changes are likely to cause HL (Table 3, online Supplementary material). In exon 5, the synonymous mutations c.918C>T (ENTREZ SNP database 1139405) and c.930C>T (ENTREZ SNP database 1135989) were heterozygously as well as homozygously present. A novel IVS1-3C>T variation was detected in one affected individual, but T is almost as frequent as C in position 3 before an exon (reviewed by Cartegni *et al*²³), suggesting that the present variation does not affect splicing of exon 2. The IVS3+13C>A and IVS5+54delG variations were identified in several affected individuals, but have previously been reported not to segregate with HL associated with the *DFNA20/26* locus.⁹ Finally, a synonymous change in the stop codon (ENTREZ SNP database 11549223) was detected.

Discussion

The present study extends the recent discovery of *ACTG1* as a new autosomal-dominant deafness gene^{8,9} by identification and characterization of a novel *ACTG1* missense mutation and the resulting HL phenotype. We also used a functional yeast assay that may help evaluate whether a missense mutation is pathogenic. Furthermore, we report a series of *ACTG1* mutation-negative families, suggesting that *ACTG1* mutations do not play a major causative role in the 20% of all hereditary HL that shows autosomal-dominant inheritance.

Impairment of actin function owing to the p.V370A mutation was demonstrated in a yeast growth assay. These experiments, however, also indicated that the mutation does not drastically disrupt actin function, as growth was only significantly impaired when the yeast were cultured at

elevated temperature or under osmotic stress. Molecular modelling supported the conclusion that the mutation does only modestly alter the function of γ -actin, as it was not predicted to globally alter the structure of γ -actin, but only affects the position of the C-terminal tail (about 6–8 residues). The effect of mutation of V370 in actins has not been reported previously, but other mutations in the C-terminal tail have been shown to alter actin function to various degrees, ranging from minor effects to lethality in yeast growth assays (reviewed by Sheterline *et al*²⁴). Many studies have suggested that the C-terminal tail of actins forms part of a binding site for important actin-binding proteins, including profilin, cofilin and gelsolin. These proteins act to either stimulate or counteract the formation of actin filaments and thereby regulate the cytoskeleton.^{24,25} In conclusion, the molecular modelling analysis therefore suggests that the p.V370A mutation may cause a modest alteration of actin-based structures, or of their dynamics of formation, in the cell.

In the cochlea, the most prominent actin-based structures are found in the hair cells.²⁶ Thus, parallel actin filaments are key structural elements in determination of the shape and function of the stereocilia of the hair cells, which are the mechanosensors for sound waves. Moreover, in the hair cells, the stereocilia are anchored into the cuticular plate, which consists of a gel-like network of actin filaments. It is therefore likely that mutations in γ -actin cause HL primarily by impairing the function and/or viability of hair cells.

Interestingly, similar to the present p.V370A mutation, the five recently reported deafness-causing mutations in γ -actin are all missense mutations that are predicted to only modestly affect the structure and function of γ -actin. The predicted modest functional impairment at the molecular level may account for the late-onset HL phenotype associated with these missense mutations in *ACTG1*. According to this hypothesis, hair cells with modestly impaired actin structures (or dynamics whereby these are formed) are fully functional, as evidenced by normal juvenile hearing of individuals with these mutations. However, such hair cells may exhibit increased sensitivity to age-dependent degeneration. As hair cells do not regenerate, this degeneration ultimately results in late-onset, progressive HL. γ -Actin is also the predominant actin in intestinal epithelial cells. In contrast to hair cells, these cells are constantly regenerating, which may explain why missense mutations that only mildly impair γ -actin function result in a non-syndromic HL phenotype. By contrast, deletion or mutations that profoundly alter the structure of γ -actin are likely to be embryonic lethal owing to lethal effects on intestinal epithelial cells and/or other cell types, which may explain why such mutations have not been reported. It should be noted, however, that any, presumably mild, gastrointestinal phenotype associated with the γ -actin deafness mutations has not been ruled out at this point.

Increased vulnerability to hair cell degeneration is the most likely cause of HL associated with missense mutations in *ACTG1*, and should indicate optimistic prognosis after cochlear implantation. The potential of this treatment may not be truly reflected in the relative poor outcome in the mutation carriers in the present study. The observation by Teig¹² of a pathological tone decay found in some frequencies where the HL was more than 50 dB, suggests, however, that missense mutations in *ACTG1* might cause degenerative lesions in the central auditory pathways in addition to hair cell degeneration. Therefore, the benefit of a cochlear implant in *DFNA20/26* patients deserves a more systematical evaluation including testing otoacoustic emissions.

All *DFNA20/26* families, including the Norwegian family, display progressive, bilateral, sensorineural HL that begins at the high frequencies.^{8,27–29} As age increases, the degree of HL increases, with threshold shifts seen at all frequencies, although a sloping configuration is usually maintained. The final outcome is most often a 'corner audiogram' and profound HL. Differences between families and intersubject are seen in average age at onset of HL, which varies from the first decade to the third decade, and rate of progression, suggesting that the different types of *ACTG1* mutations and/or differences in genetic and environmental background modify the phenotypes. In the Norwegian family, most of the hearing-impaired family members needed a hearing aid by age 20 years. The typical age of onset of HL is thus similar to three of the previously published *DFNA20/26* families, where the hearing impairment was noticed in the second decade. In contrast, one family described by Kemperman *et al*²⁹ displayed a more severe phenotype as the HL by age 15–20 years had become profound at 4–8 kHz, and by age 30–50 years, profound also at frequencies between 2 and 4 kHz. The maximum rate of progression occurred between 10 and 35 years of age with a progression rate of 3–8 dB/year, and by age 30–50 years the final degree of residual hearing was reached.²⁹

As *ACTG1* missense mutations appear to sensitize hair cells to age-dependent degeneration, it can be speculated that such mutations may be involved in the widely occurring HL of the elderly, called presbycusis. However, despite similarities between presbycusis and γ -actin-related hearing abnormality, the age of onset of γ -actin-related hearing abnormality was 30 years earlier than presbycusis, the rate of progression was faster and the degree to which mid-frequencies became affected with increasing age was much more pronounced.²⁹ Moreover, the speech recognition was found to be better in *DFNA20/26* individuals than in individuals with presbycusis.²⁹ These discrepancies may indicate that *ACTG1* missense mutations may not be involved in presbycusis to any significant extent.

The recent and present demonstrations of HL-causing mutations in *ACTG1* in a total of six families originating from Europe and USA justified examination whether

mutations in *ACTG1* are a frequent cause of autosomal hearing impairment. However, we found no *ACTG1* mutations in 19 Norwegian and Danish families segregating autosomal-dominant hearing impairment. Thus, although the number of families studied was relatively small, the present findings do not indicate that *ACTG1* mutations are a predominant cause of hereditary hearing impairment with autosomal-dominant inheritance. Thus, so far, no single gene is reported to be responsible for any substantial fraction of autosomal-dominant hearing impairment.

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