Aberrant COL11A1 splicing causes prelingual autosomal dominant nonsyndromic hearing loss in the DFNA37 locus

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Abstract

Alpha-chain collagen molecules encoded by genes that include COL11A1 are essential for skeletal, ocular, and auditory function. COL11A1 variants have been reported in syndromes involving these organ systems. However, a description of the complete clinical spectrum is lacking, as evidenced by a recent association of autosomal dominant nonsyndromic hearing loss due to a splice-altering variant in COL11A1, mapping the DFNA37 locus. Here, we describe two German families presenting prelingual autosomal dominant nonsyndromic hearing loss with novel COL11A1 heterozygous splice-altering variants (c.652-1G>C and c.4338+2T>C) that were molecularly characterized. Interestingly, the c.652-1G>C variant affects the same intron 4 canonical splice site originally reported in the DFNA37 family (c.652-2A>C) but elicits a different splicing outcome. Furthermore, the c.4338+2T>C variant originated de novo. We provide clinical and molecular genetic evidence to unambiguously confirm that COL11A1 splice-altering variants cause DFNA37 hearing loss and affirm that COL11A1 be included in the genetic testing of patients with nonsyndromic deafness.

KEYWORDS autosomal dominant hearing loss, COL11A1, DFNA37, nonsyndromic hearing loss, splice-site altering variant

Hereditary hearing loss is a clinically and genetically heterogeneous disorder. Autosomal dominant nonsyndromic hearing loss (ADNSHL) is present in roughly 20% of hearing-impaired individuals. More than 20% of genes exhibit pleiotropy, whereby variants in a single gene can be associated with syndromic or nonsyndromic hearing loss and can follow an autosomal dominant or recessive inheritance pattern (Vona et al., 2020).

The gene COL11A1 (collagen type XI alpha-1 chain; MIM #120280) is associated with autosomal dominant Marshall syndrome (MRSHS) and autosomal dominant or recessive Stickler syndrome.
type II (STL2), as well as autosomal recessive fibrochondrogenesis (FBCG1). Each of these syndromes has a phenotypic overlap that includes skeletal abnormalities and dysmorphic features, as well as variable cleft palate, ocular, and auditory phenotypes that can include mild-to-moderate hearing loss and outer ear malformations. Recently, the gene COL11A1 has been associated with ADNSHL (DFNA37; MIM #618533) through the genetic analysis of a large European-American family presenting a novel splice-site altering variant (Booth et al., 2019).

Written informed consent was obtained from participants (Approval of the University of Tübingen Ethics Commission; Nos.: 016/2014BO1 and 197/2019BO1). Medical history excluded acquired forms of hearing loss. The 37-year-old proband in Family 1 (III:3) (Figure 1a), presents stable, down-sloping, moderate-to-severe, high-frequency sensorineural hearing loss (Figure 1c), as well as hypothyroidism and diabetes. The speech discrimination with regard to monosyllables was 70% and 50% at 65-dB hearing level (HL) on the right side and 80% and 80% at 65 dB(HL) on the left side when evaluated at 34 and 37 years of age, respectively. Regarding the speech recognition threshold (SRT), the proband achieved a score of 35- and 32.5-dB hearing loss (“a1 value”) on the right and 27- and 30-dB hearing loss on the left ear at 34 and 37 years of age, respectively. He has worn hearing aids since age 6 years. His daughter (Family 1, IV:1) is currently 6.9 years old and was born after an unremarkable pregnancy and delivery. She failed newborn hearing screening but passed follow-up testing. At the age of 2 years, she was diagnosed with severe hearing loss and has used hearing aids since diagnosis. Serial audiometry has revealed stable, moderate-to-severe, high-frequency sensorineural hearing loss (Figure 1c). Her monosyllable discrimination was 75% and 65% at 65 dB(HL) on the right and 65% and 100% at 65 dB(HL) on the left side at 4.8 and 6.9 years, respectively. SRT was 27 and 30 dB hearing loss on the right, 25 and 27.5 dB hearing loss on the left at 4.8 and 6.9 years, respectively. Syndromic features including myopia, retinal detachment, midface hypoplasia, submucous cleft palate, and arthritis/joint pain have been excluded in both affected individuals in Family 1 (III:3 and IV:1). The 31-year-old proband (III:3) in Family 2 (Figure 1b) presented moderate sensorineural hearing loss in the mid- and high-frequencies since early childhood (Figure 1d) and she has worn hearing aids since age 27 years. Her pure-tone audiograms showed nearly identical thresholds taken 5 months apart at the age of 28 years (data not shown). She does not have a severe form of hearing loss that might be suggestive of progression. Her monosyllable recognition was 80% at 65 dB(HL) (SRT 20-dB hearing loss) on the right ear and 75 dB(HL) (SRT 25-dB hearing loss) on the left ear. She had a normal tympanogram and otoscopy exam. Two of her grandparents wear hearing aids. Her parents underwent pure-tone audiometry testing and had normal thresholds. Her son (Family 2, IV:1; Figure 1b) was born at term after an unremarkable pregnancy and failed newborn hearing screening. Evoked response audiometry showed mild hearing loss (Figure 1d). Transient evoked otoacoustic emissions were bilaterally absent. Distortion product otoacoustic emissions were absent on the right side and partially evocable on the left side. At the last evaluation at the age of 3.5 years, he had mild hearing loss.

**FIGURE 1** Pedigree and Sanger sequencing result in (a) Family 1 and (b) Family 2 showing segregation of the c.652-1G>C and c.4338+2T>C variants, respectively. Mutant alleles are represented with “−” and the reference allele is shown with “+.” Sanger electropherograms of the variant are shown to the right of each pedigree. Audiometry of right and left ears from (c) individuals III:3 and IV:1 in Family 1 and (d) individual III:3 in Family 2, represented as pure-tone air-conduction thresholds, as well as evoked response audiometry from individual IV:1 in Family 2 (d). Thresholds are shown with circles and crosses/vertical rectangles, for right and left ears, respectively.
bilateral sensorineural hearing loss and no other abnormalities. He was too young to undergo speech audiometry. The mother (Family 2, III:3) reported a cleft lip and palate, an occurrence not previously reported with STL2 and assumed to be due to other genetic or multifactorial causes. She reported no other abnormalities. Submucous cleft palate in her son (Family 2, IV:1) has been ruled out. Ophthalmic examination excluded amblyopia, strabismus, and phoria and confirmed normal vision. Affected individuals in both families do not report otorrhea, otalgia, tinnitus, or vertigo. Bone conduction thresholds from individuals III:3 and IV:1 in Family 1 and III:3 in Family 2 confirm sensorineural hearing loss (Figure S1).

Blood samples were collected from affected (III:3 and IV:1 of Family 1, as well as III:3 and IV:1 of Family 2) and unaffected (III:4 from Family 1 and II:1 and II:2 from Family 2) individuals in two unrelated German families (Figure 1a,b). The genomic DNAs from individuals III:3 and IV:1 in Family 1 and III:3 in Family 2 were subjected to a custom-designed high-throughput sequencing panel that included 164 hearing loss genes (HiSeq4000; Illumina Inc.). Duplication/deletion analysis of GJB6, WFS1, and POUSF4 was carried out using MLPA (P163-D1-V16; MRC Holland).

Two novel heterozygous variants in COL11A1 were identified that each disrupt canonical splice sites: NM_080629.2:c.652+1G>C (ClinVar Accession: RCV000487702.2; LOVD Genomic Variant Accession: 0000686100) in Family 1 and NM_080629.2:c.4338+2T>C (ClinVar Accession: RCV000585624.2; LOVD Genomic Variant Accession: 0000686100) in Family 2 in introns 4 and 57, respectively (Table S1). The c.652+1G>C variant in Family 1 segregated in two generations (Figure 1a), while the c.4338+2T>C variant was suspected de novo origin in the proband and transmitted to her hearing-impaired son (Figure 1b). Paternity testing of the mother was not possible to perform for definitive confirmation of the de novo status. An in vitro splicing assay was carried out as previously described (Doll et al., 2020; Tompson & Young, 2017) using amplified genomic DNA of the probands (Family 1 III:3: 1835-bp amplified genomic DNA length and Family 2 IV:1: 803-bp amplified genomic DNA length) and a normal hearing control. The c.652-1C variant impacts splicing through the loss of an acceptor site and activation of two cryptic splice acceptor sites in exon 5 that cause in-frame deletions (Figure 2a-c). TA cloning, followed by reverse-transcription polymerase chain reaction and Sanger sequencing of complementary DNA (cDNA) amplicons with the patient variant shown that 67% (28 out of 42 analyzed clones) of the amplicons utilized the first cryptic splice site (r.652-663del, p.(Gly218_Gln221del)) and 33% (14 out of 42 analyzed clones) of the amplicons used the second cryptic acceptor site (r.652-666del, p.(Gly218_Gln222del); Figure 2b and Table S2). The c.4338+2C variant leads to three abnormally spliced amplicons that include the activation of two cryptic splice donor sites in intron 57 (r.4338_4339ins4338+1_4338+4, p.(Gly1447A-Xaa12)); r.4338_4339ins4338+1_4338+30, p.(Gly1447Aals*9)) and the skipping of exon 57 (r.4285_4338del, p.(Gly1429_Met1446del); Figure 2d-f). TA cloning and follow-up testing as described above of cDNA amplicons with the patient variant indicated that 87% (40 out of 46 analyzed clones) of the amplicons showed evidence of a skipped exon 57, 6.5% (3 out of 46 analyzed clones) used the first cryptic donor site (r.4338_4339ins4338+1_4338+4) and 6.5% (3 out of 46 analyzed clones) used the second cryptic donor site (r.4338_4339ins4338+1_4338+30) (Figure 2e and Table S2).

More than 60 different variants including frameshift, splicing, missense, in-frame, and even two synonymous variants have been classified as pathogenic in COL11A1 and reported in MRSHS, STL2, and FBCG1 (deafnessvariationdatabase.org; Azaiez et al., 2018); however, only one molecularly characterized splice-altering variant has been associated with ADNSHL (Booth et al., 2019). In line with this study, our findings confirm COL11A1 splice-altering variants as causally implicated in DFNA37. Interestingly, the c.652-1G>C variant in Family 1 resides in the same canonical splice site as the c.652-2A>C variant previously reported by Booth et al. (2019) that associated COL11A1 with ADNSHL. The c.652-2A>C variant created a leaky acceptor splice site that produced a normally spliced transcript in addition to a transcript with exon 5 skipping. It was suggested that the variant may result in the loss of key N-terminal propeptide regulatory sequences of COL11A1. In vitro testing of the c.4338+2T>C variant in intron 57 disclosed skipping of exon 57 and the use of two cryptic donor sites with an in-frame and out-of-frame consequence. Considering the clinical presentation of our patients with the molecular characterization of splice variants that revealed multiple in-frame mutant transcripts, we hypothesize that these in-frame variants may lead to a milder phenotype (DFNA37) due to partial residual function of the protein (i.e., noncomplete loss-of-function alleles), whereas those with more severe phenotypes may be due to frameshift, truncating loss-of-function alleles, and missense variants which induce splice altering effects or substitution of glycine in a repeat Gly-Xaa-Yaa region. Interestingly, one additional variant was recently described in a Czech family (NM_080629.2:c.1560delC) with ADNSHL. The variant was described as a splice-altering variant but a splicing assay was not performed and in silico tools did not predict a splicing effect (Čopíková et al., 2020). Functional characterization of this variant will be important for a refined genotype-phenotype correlation due to variants in COL11A1.

The family described by Booth et al. (2019) demonstrated bilateral, postlingual, slowly progressive, and mild-to-moderate sensorineural hearing loss without any other symptoms. Consistent with this report, the two families manifested bilateral sensorineural hearing loss without other symptoms. The thresholds from individuals III:1 and IV:1 in Family 1 were both either normal or indicate a mild hearing loss in low frequencies and were sloping to a moderate-to-severe hearing loss in high frequencies (Figure 1c). Importantly, the hearing loss in the affected individuals in Family 1 is stable and at last measurement, there was no evidence of progression to a severe hearing loss in Family 2 individual III:3. The thresholds from the individual III:3 in Family 2 were normal to 1 kHz, then increased to show moderate hearing loss until 4 kHz and slightly increased at 6 and 8 kHz (Figure 1d). Newborn hearing screening of individuals IV:1 in both families confirmed one individual with congenital onset (Family 2, IV:1), which is the first reported congenital onset with COL11A1-associated ADNSHL and
FIGURE 2 (See caption on next page)
also demonstrates that variants in this gene can cause prelingual deafness, as documented in all affected individuals in this study.

Although the precise mechanism of COL11A1-associated hearing impairment has not been elucidated, disruption of COL11A1 is consistently associated with hearing loss, as demonstrated by about 84% of individuals diagnosed with MRSHS having hearing loss, which supports the critical function of this protein in the auditory system (Bacciu et al., 2018). It also demonstrated that the source of COL11A1 mRNA is in the tectorial membrane and suggested its mutation affects normal cochlear function (Shpargel et al., 2004). Moreover, the pleiotropy exhibited by this gene, like other genes that are associated with syndromic and nonsyndromic hearing loss, remains to be fully characterized.

In summary, we report on and characterize two novel splice-altering variants associated with DFNA37, providing confirmatory evidence of COL11A1 as a bona fide ADNSHL gene. We recommend COL11A1 be included in the routine diagnostic testing of patients with both syndromic and nonsyndromic forms of deafness.

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AUTHOR CONTRIBUTIONS

Saskia Biskup is an owner of CeGaT. The remaining authors declare that there are no conflict of interests.

Barbara Vona submitted variants to clinical databases. Aboulfazl Rad and Barbara Vona provided functional experiments and wrote the first manuscript draft. Grigoriy A. Yanus and Evgeny N. Imyanitov validated splicing nomenclature, performed the literature review, and curated genetics data. Thore Schade-Mann, Philipp Gamerdinger, Marcus Müller, Hubert Löwenheim, and Anke Troplitzsch recruited the families, and curated and interpreted clinical data. Thore Schade-Mann performed clinical re-examination. Anke Troplitzsch, Hubert Löwenheim, and Barbara Vona conceived the study. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author. The genetic data are not publicly available due to data privacy or ethical restrictions. COL11A1 variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/?term=COL11A1) with accession IDs RCV000487702.2 (NM_080629.2:c.652-1G>C) and RCV000585624.2 (NM_080629.2:c.4338+2T>C). Additionally, they were deposited in LOVD v.3.0 (https://databases.lovd.nl/shared/genes/COL11A1) under genomic variant accession nos. 0000686099 (NM_080629.2:c.652-1G>C) and 0000686100 (NM_080629.2:c.4338+2T>C).

LIST OF WEB RESOURCES AND URLS

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
Deafness Variation: databasedeafnessvariationdatabase.org
LOVD v.3.: https://www.lovd.nl/3.0/home
OMIM: https://www.omim.org/clinvar

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FIGURE 2  (a) Gel electrophoresis of the reverse-transcription polymerase chain reaction (RT-PCR) from the wild-type control, the c.652-1C variant, and empty pSPL3 vector amplicons, as well as transfection-negative and PCR-negative controls. (b) The vector construct of the in vitro splice assay illustrates the wild-type or mutant amplicons inserted between exons A and B of the pSPL3 vector with a splicing schematic of the c.652-1C variant (upper) and wild-type allele (lower). The wild-type (upper left sequencing panel) shows expected splicing. The c.652-1C variant activates two cryptic acceptor splice sites (r.652-663del and r.652-666del) in exon 5 (upper right sequencing panels). The empty vector control (lower sequencing panel) performed as expected. (c) In silico splice predictions of the wild-type (top, red G) and c.652-1G>C (bottom, red C) marked with black arrows. Cryptic splice acceptor sites that are activated due to the variant are underlined and marked with red arrows and validated with an in vitro splice assay and TA cloning. (d) Gel electrophoresis of the RT-PCR from the wild-type control, the c.4338*2C variant, and empty pSPL3 vector amplicons. The transfection and PCR-negative controls performed as expected. (e) The vector construct of the in vitro splice assay illustrates the wild-type or mutant amplicons inserted between exons A and B of the pSPL3 vector with a splicing schematic of three splice products (r.4338_4339ins4338+1_4338+4, r.4338_4339ins4338+1_4338+30, and r.4285_4338del) due to the c.4338+2C variant (upper) and wild-type allele (lower). The wild-type (upper left sequencing panel) shows the expected splicing. The c.4338+2C variant activates two cryptic acceptor splice sites in intron 57 (upper right sequencing panels) and evokes skipping of exon 57 (lower sequencing panel) that was the same sequence as the empty vector control (lower sequencing panel). (f) In silico splice predictions of the wild-type (top, red T) and c.4338+2T>C (bottom, red C) marked with black arrows. Cryptic splice donor sites that are activated due to the variant are underlined and marked with red arrows and validated with an in vitro splice assay and TA cloning. Var, variant; WT, wild type.
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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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