A Novel de novo Mutation in *CEACAM16* Associated with Postlingual Hearing Impairment

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Key Words

Autosomal dominant nonsyndromic hearing loss · *CEACAM16* · De novo mutation · *DFNA4B* · Next-generation sequencing · Parent-child trios

Abstract

Mutations in *CEACAM16* cause autosomal dominant nonsyndromic hearing loss (DFNA4B). So far, 2 families have been reported with segregating missense mutations, both in the immunoglobulin constant domain A of the *CEACAM16* protein. In this study, we used the TruSight One panel to investigate a parent-child trio without familial history of hearing loss and one affected child. When filtering for recessive inheritance and de novo events, we discovered a de novo *CEACAM16* mutation (c.1094T>G, p.Leu365Arg) as the sole likely pathogenic variant. The de novo mutation was confirmed by Sanger sequencing and STR analysis. The proband's hearing loss closely matches the described onset and severity for DFNA4B. We present the third *CEACAM16* variant and the first de novo mutation in *CEACAM16*. This de novo mutation is robustly described as a pathogenic mutation according to in silico mutation prediction tools and affects a highly conserved amino acid in the most strongly conserved *CEACAM16* N2 domain. Our strategy of screen-

ing family trios enhances de novo mutation discovery and the exclusion of other variants of potential interest through pedigree filtering.

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The complex structural and functional relationships between the various components of the ear are indispensable for proper hearing. One of the most important sensory components, the cochlea, is where the mechanically detected sound waves are transformed into neurological signals for audiological cognition. The cochlea contains a specialized structure called the tectorial membrane. While the function of the tectorial membrane is not yet completely understood, it has a frequency-dependent character that is influenced by its architecture and is composed of multiple specialized proteins such as 3 genetically diverse collagens type II, IX and XI as well as noncollagenous proteins like otogelin (OTOG), otogelin-like (OTOGL), otolin 1 (OTOL1), tectorin alpha (TECTA), tectorin beta (TECTB), and carcinoembryonic antigen cell adhesion molecule 16 (*CEACAM16*) [Legan et al., 2014; Jones et al., 2015]. Impaired OTOG and OTOGL function causes autosomal recessive nonsyndromic hearing loss [Schraders et al., 2012; Yariz et al., 2012], where-

Fig. 1. Schematic overview of CEACAM16 and mutational analysis. **a** CEACAM16 has 7 exons, including one noncoding exon 1. The known mutations are in the fourth exon (pink), whereas the novel mutation of the index is in exon 6 (turquoise). **b** The amino acid sequence per exon is represented within each dashed boundary. The red amino acid represents overlapping amino acids between adjacent exons. Changes of the single amino acids by mutations are

marked in pink for known mutations and turquoise for our investigated mutation. **c** The amino acid sequence corresponding to each protein domain is demarcated by solid lines for the N1 domain (green), A domain (blue), B domain (purple) and N2 domain (red). The position of each of the 3 CEACAM16 variants is marked in each of the corresponding domains. CEACAM16 domain and exon boundaries were prepared using Ensembl release 75.

as mutations in CEACAM16 have been associated with autosomal dominant nonsyndromic hearing loss [Zheng et al., 2011]. TECTA is responsible for both autosomal dominant and recessive inherited hearing loss [Verhoeven et al., 1998; Mustapha et al., 1999].

Autosomal dominant hearing loss occurs in ~20% of all cases with a genetic background [Petersen, 2002]. Until now, there are around 50 deafness loci identified with over 30 genes known [http://hereditaryhearingloss.org]. CEACAM16 (OMIM 614591) maps to the DFNA4 locus on chromosome 19q13.32, which is subdivided into DFNA4A (OMIM 600652) described by mutations in MYH14 (OMIM 608568) and DFNA4B (OMIM 614614) caused by mutations in CEACAM16 [Zheng et al., 2011]. CEACAM16 possesses 7 exons (fig. 1a) and codes for a 425 amino acid protein (fig. 1b). The protein has 4 domains: one NH₂-terminal N1 immunoglobulin variable domain, followed by 2 immunoglobulin constant-like domains A and B, and one COOH-terminal N2 domain with immunoglobulin variable-like character (fig. 1c). Among the CEA gene family, CEACAM16 is the most conserved gene in mammals and has a carboxyl-terminal N2 domain that is especially well conserved [Kammerer and Zimmermann, 2010]. The secreted protein is expressed in interdental and Deiters cells as well as in the tectorial membrane. In the mouse, CEACAM16 expres-

sion was first observed by postnatal days 12 and 15, coinciding with the initial onset of hearing. Ceacam16 knockout mice demonstrated high and low frequency hearing impairment. Young mice had normal hearing until 1–2 months of age that progressed throughout life [Kammerer et al., 2012]. These observations mirror postlingual progressive hearing loss that corresponds closely to the described DFNA4B hearing loss in an American family (1070) [Zheng et al., 2011]. Linkage analysis of this family localized to chromosome 19q13 [Chen et al., 1995]; however, this family was negative for a mutation in MYH14, the previously identified gene in the DFNA4 locus. A missense mutation (c.418A>C, p.Thr140Pro) in a neighboring gene, CEACAM16, was disclosed that led to the first association of autosomal dominant hearing loss with this gene [Zheng et al., 2011]. Recently, a Chinese family was detected with a segregating CEACAM16 missense mutation (c.505G>A, p.Gly169Arg) with a slightly different phenotype that showed increased hearing thresholds in high frequencies [Wang et al., 2015]. In this report on a German boy with postlingual hearing impairment, we provide the third missense mutation in CEACAM16 (c.1094T>G, p. Leu365Arg) and the first de novo mutation in this gene that is unanimously predicted with a likely pathogenic outcome using in silico mutation pathogenicity prediction tools.

Fig. 2. Familial pedigree, Sanger sequence confirmation and audiogram profile in the investigated index. **a** Pedigree of the investigated index. Parents and an older sibling are healthy with normal hearing. There is no family history of hearing loss. **b** Validation by Sanger sequencing. Both parents and the sibling show the WT se-

quence in the c.1094 position (left). The heterozygous c.1094T>G mutation was confirmed in the index (right). **c** The child has a bilateral hearing loss that moderately affects all frequencies except 0.125 kHz.

Materials and Methods

Analysis with Targeted Next-Generation Sequencing

Genomic DNAs from the proband and his parents were extracted from whole blood using a standard salt extraction method. The index did not disclose pathogenic mutations in established diagnostic screening for *GJB2*, *GJB6*, and *STRC*. The proband was included in our hearing loss next-generation sequencing (NGS) study, wherein we analyzed the index and his parents using the TruSight One panel according to manufacturer's instructions (Illumina, San Diego, Calif., USA). The TruSight One is a commercial NGS panel for the targeted genomic enrichment of 4,813 genes including around 100 hearing loss genes. The samples were paired-end sequenced on a MiSeq desktop sequencer and mapped to the human genome reference sequence (GRCh37, hg19) (Illumina). The generated bam files were loaded in GensearchNGS software (PhenoSystems SA, Wallonia, Belgium). Filtering criteria included coverage >20 reads, variant frequency >20%, minor allele frequency <0.01, and selected for mutation type such as missense, nonsense, synonymous, and splice variants. The variants passing these conditions were further filtered by pedigree using parental variants. As this case was initially presumed with a recessive mode of inheritance from pedigree analy-

sis (fig. 2a), variants were investigated which were heterozygous in the parents and homozygous or compound heterozygous in the index. These initial analyses were unable to yield a positive result; therefore, supporting de novo mutation filtering in the proband. All filtered variants were investigated in Alamut visual version 2.6.0 (Interactive Biosoftware, Rouen, France) and queried by the bioinformatics tools FATHMM [Shihab et al., 2013], SIFT [Ng and Henikoff, 2001], MutationAssessor [Reva et al., 2011], MutationTaster [Schwarz et al., 2014], and PolyPhen-2 [Adzhubei et al., 2010]. Splice site predictions were performed using the Alamut tools SpliceSiteFinder-like, MaxEntScan [Yeo and Burge, 2004], NNSPLICE [Reese et al., 1997], GeneSplicer [Pertea et al., 2001], and Human Splicing Finder [Desmet et al., 2009]. Alamut simultaneously disclosed amino acid and nucleotide (phyloP) conservation. Lastly, databases such as OMIM [http://www.ncbi.nlm.nih.gov/omim], MGI [Smith et al., 2014], UniGene [http://www.ncbi.nlm.nih.gov/unigene/], HGMD [Stenson et al., 2014], LOVD [Fokkema et al., 2011], Deafness Variation Database (DVD) [Shearer et al., 2014], ClinVar [http://www.ncbi.nlm.nih.gov/clinvar/], Exome Variant Server [http://evs.gs.washington.edu/EVS/], and ExAC [http://exac.broadinstitute.org/] were used for gene, gene expression, and mutation background information.

Validation of the de novo Mutation by Sanger Sequencing and STR Analysis

For validation of the *CEACAM16* (NM_001039213) c.1094T>G variant, primers were designed using Primer3 [Untergasser et al., 2012], which amplified a 352-bp amplicon from genomic DNA. Standard PCR cycling conditions were used with the forward 5'-AGATGCAGACCAAACTGACC-3' and reverse primer 5'-CT-TCCAGTGTCTCAGTCTTGC-3' (Metabion, Martinsried, Germany). Sanger sequencing of the amplicons followed using an ABI3130xl 16-capillary sequencer (Life Technologies, Carlsbad, Calif., USA). The parents, sibling, index, and 50 controls were sequenced. For confirming the de novo event, a PowerPlex 5 (Promega, Madison, Wis., USA) test of the proband and his parents was performed, using 4 highly polymorphic markers from different chromosomes and 1 marker for the sex.

Results

Clinical Information

After an uncomplicated pregnancy, the male proband was born at 39 gestational weeks to unrelated German parents who were 29 (mother) and 34 (father) years of age. At birth, nuchal cord was noted and the proband measured APGAR scores of 10 each at 1 and 5 min. The proband showed indication of hearing impairment with a conspicuous speech audiogram at ~9 years of age, 2 years before bilateral sensorineural hearing loss at the age of 11 could be detected (fig. 2c). His hearing tested normally at the age of 10. Speech testing showed dyslalia that improved with speech therapy; however, the index has developed normal speech and language skills and benefits from ongoing speech therapy. Since primary school, he has had migraine episodes occurring together with stomach ache, dizziness, and sickness. These all improved except for migraines after he began wearing glasses. As the dizziness and sickness subsided, diagnostic vestibular function testing was not performed. Physiotherapy and medication have aided his migraines. Furthermore, he has seasonal and food allergies as well as severe neurodermatitis and asthma. Migraine and seasonal allergies appear on his maternal side. Prior to developing hearing impairment, he reported noise sensitivity and headaches that correlated with strained hearing. His audiogram exhibits a stable flat threshold throughout all frequencies except 0.125 kHz in the 40–60 dB range (fig. 2c). His hearing loss has not progressed in the short span of one year since his diagnosis. No episodes of tinnitus were reported. Additionally, transient-evoked otoacoustic emissions (TEOAEs) and distortion product otoacoustic emissions (DPOAEs) were absent. TEOAE tests were recorded with 9 and 44% reproduc-

ibility for right and left ears, respectively, and the total OAE response was lower than the noise floor. In the DPOAE tests, the signal-to-noise ratios over all frequencies yielded a negative result. Auditory brainstem response and auditory steady-state response measurements showed elevated thresholds which coincided with the pure tone audiometry results and confirmed a cochlear site of lesion. EEG was normal. He was treated with hearing aids at the age of 11 and attends a normal school. There is no evidence of developmental delay and a suspicion of dyslexia was excluded. His blood profile was unremarkable. Cytomegalovirus, varicella zoster virus and toxoplasma infections were excluded at 11 years of age. His parents have normal hearing, and there is no familial history known (fig. 2a). Based on the relatively high proportion of *GJB2*, *GJB6*, and *STRC* mutations contributing to autosomal recessive hearing loss, these genes were tested in routine diagnostics without pathological findings.

NGS Using the TruSight One Panel and Bioinformatics Analysis

The proband and his parents were sequenced in parallel with a MiSeq desktop sequencer, each generating over 20 million aligned sequences and around 70,000 variants. The first analysis filtered for a recessive mode of inheritance and yielded negative results with exclusion of all variants by filtering or not meeting analysis criteria. Therefore, all variants in common between the proband and his parents were excluded, allowing for the detection of a heterozygous de novo mutation (c.1094T>G, p.Leu365Arg) in the gene *CEACAM16* (fig. 2b). The gene had an average of over 80-fold coverage in all exons except exon 1, which is noncoding and meets quality, coverage and variant frequency criteria.

The mutation was analyzed using the pathogenicity prediction programs FATHMM, SIFT, MutationAssessor, MutationTaster, and PolyPhen-2. FATHMM shows a deleterious score of roughly 0.80 for the coding c.1094T>G mutation. The mutation is predicted as deleterious and disease causing in SIFT (score: 0.02, median: 3.40) and MutationTaster (p value: 0.707), respectively (table 1). In contrast to MutationAssessor, presenting a neutral functional impact and affected protein binding site, PolyPhen-2 predicted the mutation as probably damaging (score: 1.0). The c.1094T>G mutation occurs in a moderately conserved nucleotide (phyloP score: 2.87). The Leu to Arg substitution appears in a highly conserved amino acid considering 19 species and occurs in the most conserved N2 domain (table 1).

Table 1. Pathogenicity prediction program and database analyses comparing the presently described mutation to the known mutations in *CEACAM16*

Prediction programs and databases	c.1094T>G	c.418A>C	c.505G>A
FATHMM	deleterious (0.98 NC, 0.80 C)	benign (0.24 NC, 0.07 C)	benign (0.21 NC, 0.05 C)
SIFT	deleterious (score: 0.02)	tolerated (score: 0.22)	tolerated (score: 0.16)
MutationAssessor	functional impact neutral, protein binding site	functional impact low	functional impact neutral
MutationTaster ^a	disease causing (p value: 0.707)	polymorphism (p value: 1)	polymorphism (p value: 1)
PolyPhen-2 ^b	probably damaging (score: 1.0)	probably damaging (score: 0.991)	possibly damaging (score: 0.642)
phyloP ^c	moderately conserved nucleotide (2.87)	weakly conserved nucleotide (0.29)	weakly conserved nucleotide (0.21)
Splice Site prediction by Alamut	no difference	small difference in GeneSplicer	new 3' splice site predicted
ClinVar	not present	pathogenic	not present
Deafness Variation Database	unknown significance	pathogenic	unknown significance
ExAC	not present	not present	not present
Exome Variant Server	not present	not present	not present
HGMD	not present	pathogenic	not present
LOVD	not present	not present	not present

FATHMM = The p values range between 0 and 1. Values over 0.5 are predicted as deleterious, p values below 0.5 are predicted as neutral or benign. The closer the value is to 0 or 1 reflects a greater confidence. SIFT Aligned Sequence tool values <0.05 predict substitutions that are deleterious, whereas values >0.05 predict benign substitutions. NC = Noncoding; C = coding.

^a MutationTaster operates on a scale from 0 to 1.0, with 1.0 having the highest probability of being benign or disease causing. ^b PolyPhen-2 scores operate on a scale from 0 to 1.0. 1.0 represents a disease causing prediction and 0 a benign prediction. ^c In phyloP, a positive score shows evolutionary conservation and a negative score shows evolutionary acceleration.

ClinVar, ExAC, Exome Variant Server, HGMD, and LOVD had no further information about this mutation. The mutation is classified in DVD with unknown significance. The mutation is not predicted to change splicing (table 1). Interestingly, the transversion results in an exchange from T to G introducing a new CpG site (fig. 2b). In our small cohort, we could not find any pathogenic changes in the gene *CEACAM16* in 19 healthy individuals.

The reported c.418A>C and c.505G>A mutations (table 1) are both predicted as benign in FATHMM, tolerated in SIFT (protein ID NP_001034302) and polymorphic in MutationTaster. PolyPhen-2 predicted the c.418A>C mutation as probably damaging (score: 0.991), and the c.505G>A mutation as possibly damaging (score: 0.642). Both mutations occur in weakly conserved nucleotides. The p.Thr140Pro substitution occurs in a highly conserved amino acid, whereas the p.Gly169Arg substitution occurs in a weakly conserved amino acid considering 19 species. Both mutations are predicted as inducing a splice difference. Most notably, the c.505G>A mutation is predicted as causing an additional 3' splice site.

Similar to our described c.1094T>G mutation, the c.505G>A mutation is not present in the databases we used for analysis except DVD, where it is classified with unknown significance. However, the c.418A>C mutation is already described in DVD, HGMD and ClinVar as pathogenic (table 1).

In summary, the identified *de novo CEACAM16* mutation c.1094T>G, p.Leu365Arg in *CEACAM16* in the proband has a stronger predicted pathogenic outcome compared to the already described mutations in the literature (table 1).

Validation of the de novo Mutation

Sanger sequencing confirmed the heterozygous c.1094T>G mutation in the index and wild-type sequences in both parents and sibling (fig. 2b). The c.1094T>G mutation was absent in 50 controls. PowerPlex5 analysis was consistent with biparental inheritance of the tested STR alleles from both parents (data not shown). These results clearly demonstrate the *de novo* event of the c.1094T>G, p.Leu365Arg mutation in the proband.

Discussion

Compatibility of the Clinical Picture

At the age of 11 years, our proband manifested a moderate sensorineural hearing loss which affected nearly all frequencies and disclosed a characteristic flat audiometric profile (fig. 2c). The phenotypes of the 2 previously described patients with *CEACAM16* variants compare well with our presented patient. The American and Chinese families are both described having binaural autosomal dominant nonsyndromic sensorineural hearing loss with an onset in the second decade of life [Zheng et al., 2011; Wang et al., 2015]. There were 2 slight differences in these families compared to our proband. Firstly, the American family had well-described progression to ~50 dB in adulthood [Zheng et al., 2011]. The audiograms currently available demonstrate stable hearing loss that will be monitored for similar progression in adulthood. Secondly, the hearing loss in the Chinese family appears in the higher frequencies, whereas the audiogram profile for our proband is flat except for 0.125 kHz [Wang et al., 2015]. A unique feature of our proband was that he exhibited noise sensitivity before the onset of his hearing loss. Mutant *Ceacam16* mouse lines that have undergone extensive hearing loss characterization provide a useful means for correlating mutations in *CEACAM16* to a human phenotype. *Ceacam16*^{-/-} mice demonstrate increasing hearing thresholds throughout their lifetime comparable to the progressive hearing loss described in the American family [Kammerer et al., 2012]. As conclusive hearing loss onset in our proband was diagnosed only one year ago, there has been limited opportunity to test for similar progression.

De novo Mutation in CEACAM16

The presently described heterozygous c.1094T>G mutation is the first reported de novo mutation in *CEACAM16*. De novo mutations are rare in the context of well-conserved genes. The mutation rate for *CEACAM16* is $10^{-4.6392}$, which is not within the limits of what would be expected for a constrained gene, but this mutation rate is in the same range such as *CRYM* ($10^{-4.9127}$) [Samocha et al., 2014]. *CRYM* is an autosomal dominant nonsyndromic deafness gene (DFNA40), in which a previously described de novo mutation was detected [Abe et al., 2003]. In an average genome, there are 74 germline de novo single nucleotide variants and approximately one de novo mutation per exome [Veltman and Brunner, 2012]. In contrast to the effect of advanced maternal age, typically with greater impact on chromo-

somal structure, the single nucleotide variant rate is heavily dependent on paternal age, making the father the predominant contributor of de novo mutations. Usually, de novo mutations have stronger disease causing potential than inherited mutations because unlike inherited mutations, de novo mutations have not undergone stringent evolutionary selection [Veltman and Brunner, 2012]. The number of paternal germline mutations increases by ~2.01 mutations per year with mutations doubling every 16.5 years. In our case, the father was around 33 years old at the time of conception. At present, this is nearly the average paternal age and increases the average mutational load for the child to ~69.9, according to a recent deCODE study [Kong et al., 2012]. Interestingly, chromosome 19, which includes *CEACAM16*, shows a greater than expected increase in the number of mutations per annual increase in paternal age, especially compared to similarly sized chromosomes such as chromosomes 21 and 22 [Kong et al., 2012]. This supports the significance of our de novo mutation.

CpG sites are often methylated and serve as a mechanism for inhibiting gene expression. Interestingly, a new CpG site originates due to the T to G transversion from the mutation. CpG sites normally have higher mutation rates due to spontaneous deamination of 5-methylcytosine that causes an exchange of C to T and results in a loss of a CpG site. This present case illustrates a converse situation with a CpG site gain [Campbell and Eichler, 2013]. The impact of this novel CpG site has not been investigated; however, one consequence of this mutation could be altered gene methylation that influences gene regulation. Additionally, under similar experimental settings and filtering parameters, there are no pathogenic mutations within *CEACAM16* in a small group of population-matched healthy individuals (n = 19). This mutation was also absent in large cohorts of control individuals such as ExAC as well as in 50 Sanger-sequenced controls for testing whether there is a minor allele frequency for the c.1094T>G mutation.

Predicted Effect of the c.1094T>G Mutation on Protein Interaction Partners and the N2 Domain

CEACAM16 has 2 primary interacting partners, *TECTA* and *TECTB* that are both important for the generation of the striated-sheet matrix and Hensen's stripe. Mouse experiments demonstrate that *TECTB* is directly influenced by *CEACAM16* because loss of *CEACAM16* reduces the level of *TECTB* and the striated-sheet matrix and Hensen's stripe remain immature [Cheatham et al.,

2014]. Additionally, TECTA is undetectable after postnatal day 22 [Rau et al., 1999]. This is a plausible explanation why hearing loss occurs at a later stage and is progressive. Moreover, this could explain why there were neither hearing nor anatomical differences between wild-type mice and *Ceacam16* heterozygotes, which were both clearly identified in homozygous mice [Cheatham et al., 2014] because the knock out mouse elicited the reduced expression that resulted in hearing loss. Furthermore, hearing loss could be influenced according to the affected domain. The c.1094T>G mutation resides in the N2 domain of CEACAM16 (fig. 1c) and is predicted by MutationAssessor to affect a protein binding site. This could be a binding site for TECTB and TECTA crosslinking with CEACAM16 for developing the striated-sheet matrix structure [Cheatham et al., 2014]. The N2 domain has homophilic interactions and forms dimers for supporting cell adhesion. It can interact in *cis* and *trans*, but will form only disulfide bridges by aligning in parallel [Kammerer et al., 2012]. The effect of the mutation on the dimerization and homophilic interactions would be interesting for further investigation. For additional genotype-phenotype correlations for mutations occurring in the various domains, more families with mutations in *CEACAM16* are required.

Interpretation of the Mutation Compared to Already Detected Mutations

In contrast to the mutations described in the literature (c.418A>C, c.505G>A) that occur in the A domain, the mutation in our proband was identified in the carboxyl-terminal N2 domain (fig. 1c). The N2 domain is the most conserved domain among the CEA gene family [Kammerer and Zimmermann, 2010]. Various prediction programs classify our mutation c.1094T>C as inducing a strong biochemical change with likely pathogenic effect (table 1), whereas the c.418A>C and c.505G>A mutations of the A domain show weak conservation and are in silico predicted as likely benign in 4 out of 5 mutation pathogenicity programs (table 1). This could be due to mutations residing in the A domain, which is less conserved and has a ubiquitous immunoglobulin constant-like domain character [Kammerer and Zimmermann, 2010]. Using our described filtering parameters, these results would have been excluded as unlikely pathogenic, and we would have had difficulties in implying their pathogenicity. Both previously identified *CEACAM16* mutations were detected using whole-genome linkage and whole-exome sequencing [Zheng et al., 2011; Wang et al., 2015]. The other variants that were also present in

the sequencing dataset were communicated for the c.418A>C variant but limited to the DFNA4 region [Zheng et al., 2011]. One of the consequences of broadly screening large subsets of genes, as in the case of clinical and whole exomes, is the generation of large numbers of variants that require interpretation including synonymous variants, which could impact splice sites [Schultz et al., 2009; Wang et al., 2015]. It is common for even healthy individuals to carry private pathogenic mutations complicating the understanding of disease causing mutations [Jang et al., 2015]. While bioinformatics analyses are an important and powerful strategy for variant interpretation, there are limitations that have not yet been overcome, including changes in pathogenicity predictions with ongoing advances in genomics. One example is the presently described SIFT score. We analyzed the reported mutation as tolerated, whereas the previous authors described the former 2 mutations as damaging. Reasons for this discrepancy could be that (1) the prediction outcome has changed, (2) the SIFT tool version has changed, or (3) an alternative protein ID was used in the analysis. As there are currently many excellent in silico tools available, querying variants with multiple programs is an approach to achieve greater understanding of predicted pathogenic outcomes of variants.

The strength of parent-child trio sequencing resides in permitting variant filtering according to pedigree as well as allowing for the rapid discovery of de novo mutations. Whole-genome linkage mapping is complicated by de novo mutations and, therefore, parent-child trios in a whole-exome sequencing approach provide the best strategy for uncovering these mutations [Ku et al., 2012].

Careful attention should be exercised when analyzing variants in *CEACAM16* for pathogenic potential as well as analyzing the impact of the mutation on the domain. In conclusion, our de novo *CEACAM16* mutation supports its association with hearing loss and confirms the autosomal dominant nonsyndromic character of *CEACAM16*.

Acknowledgments

The authors would like to express their sincere gratitude to the family for their participation in this study. Additionally, we thank Dr. Marcus Ditttrich, Dr. Tobias Müller, Beat Wolf, Dr. Andrea Gehrig, and Dr. Simone Rost for their expert advice.

Statement of Ethics

This study was approved by the Ethics Committee of the University of Würzburg. Informed written parental consent was obtained prior to initiating our investigation.

Disclosure Statement

The authors have no conflicts of interest to declare.

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