Disruption of the *ATE1* and *SLC12A1* Genes by Balanced Translocation in a Boy with Non-Syndromic Hearing Loss

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Key Words

ATE1 · Disease-associated balanced chromosome rearrangement · Non-syndromic hearing impairment · Reciprocal translocation · *SLC12A1*

Abstract

We report on a boy with non-syndromic hearing loss and an apparently balanced translocation t(10;15)(q26.13;q21.1). The same translocation was found in the normally hearing brother, father and paternal grandfather; however, this does not exclude its involvement in disease pathogenesis, for example, by unmasking a second mutation. Breakpoint analysis via FISH with BAC clones and long-range PCR products revealed a disruption of the arginyltransferase 1 (*ATE1*) gene on translocation chromosome 10 and the solute carrier family 12, member 1 gene (*SLC12A1*) on translocation chromosome 15. SNP array analysis revealed neither loss nor gain of chromosomal regions in the affected child, and a targeted gene enrichment panel consisting of 130 known deafness genes was negative for pathogenic mutations. The expression patterns in zebrafish and humans did not provide evidence for ear-specific functions of the *ATE1* and *SLC12A1* genes. Sanger sequencing of the 2 genes in the boy and 180 *GJB2* mutation-negative hearing-impaired individuals did

not detect homozygous or compound heterozygous pathogenic mutations. Our study demonstrates the many difficulties in unraveling the molecular causes of a heterogeneous phenotype. We cannot directly implicate disruption of *ATE1* and/or *SLC12A1* to the abnormal hearing phenotype; however, mutations in these genes may have a role in polygenic or multifactorial forms of hearing impairment. On the other hand, it is conceivable that our patient carries a disease-causing mutation in a so far unidentified deafness gene. Evidently, disruption of *ATE1* and/or *SLC12A1* gene function alone does not have adverse effects. © 2013 S. Karger AG, Basel

Autosomal reciprocal translocations affect approximately 0.1% of newborns [Hook and Hamerton, 1977]. When associated with abnormal phenotypes and/or developmental delay, apparently balanced chromosome rearrangements pose challenging situations in genetic counseling. Gene disruption by translocation or micro-deletions/duplications in the breakpoint region or elsewhere in the genome may have phenotypic consequences. The latter are more frequent in de novo translocations with syndromic phenotypes, whereas gene disruption appears to occur at comparable frequencies in phenotypi-

cally normal and abnormal translocation carriers [Baptista et al., 2008]. This argues in favor of the notion that in many cases gene disruption can be compensated for and has only mild or no phenotypic expression. It has been suggested that the breakpoints in non-syndromic patients may be associated with common and complex diseases [Bache et al., 2006]. Chromosomal rearrangements can cause haploinsufficiency not only by direct gene disruption, but also by position effects separating the coding region of a gene from regulatory elements occurring at a considerable distance as great as one megabase. This event may consequently lead to gene dysregulation [Kleinjan and van Heyningen, 2005]. Disease-associated balanced chromosome rearrangements provide a powerful tool for the identification of disease-causing genes [Bugge et al., 2000; Higgins et al., 2008].

If a balanced rearrangement is detected both in the carrier parent and offspring, then the risk for phenotypic abnormality is generally thought to be very low. Nonetheless, there are several studies reporting patients with abnormal phenotypes despite having the same balanced rearrangement as their phenotypically normal carrier parent [Warburton, 1991]. One possible explanation for discordant phenotypes in carriers of the same familial translocation or inversion is segregation of a second genetic mutation on the homologous chromosomes (compound heterozygosity) or elsewhere in the genome (digenic/polygenic inheritance). Furthermore, additional cryptic genomic imbalances may arise in the germline of balanced translocation carriers due to non-homologous recombination events.

We identified a family with a balanced reciprocal translocation t(10;15)(q26.13;q21.1) that was inherited through 3 generations. All translocation carriers were healthy with no reported abnormalities except non-syndromic sensorineural hearing loss affecting one child. Hearing impairment is estimated to affect one out of every 1,000 newborns and increases to 3.5 out of 1,000 individuals by 18 years of age [Morton and Nance, 2006]. Furthermore, it is hypothesized that up to 1% of the greater than 20,000 genes in humans are necessary for hearing [Friedman and Griffith, 2003]. If roughly 200 genes are anticipated to play various roles in hearing and given that the number of known deafness genes (<http://hereditary-hearingloss.org>) are fewer than the suspected number, then there are many more deafness genes remaining to be discovered. We analyzed the chromosome breakpoint regions of our proband with the idea to identify a candidate gene(s) for hearing impairment. Previous studies have already demonstrated that in a given patient/family, dis-

ease-associated balanced chromosome rearrangements can provide a visible bridge between hearing impairment and the underlying genotype [Williamson et al., 2007; Damatova et al., 2009; Schneider et al., 2009].

Material and Methods

Classic and Molecular Cytogenetic Analyses

Cytogenetic analyses were performed on the index patient as well as his parents, paternal grandparents and brother from peripheral blood lymphocyte cultures using conventional GTG-banding techniques at the 550-band level.

To delineate the breakpoint regions, FISH was carried out using selected BAC probes from the chromosomal 10q25.3q26.13 and 15q15q21.3 regions (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000355443). The locations of these BAC clones were chosen from the Ensembl browser (GRCh37) and ordered from the Children's Hospital Oakland Research Institute (Oakland, Calif., USA). In order to amplify larger BAC subfragments ranging from 3.5 to 10.7 kb, the Expand Long Template PCR System (Roche Diagnostics, Mannheim, Germany) was used according to the manufacturer's recommendations with a series of primer pairs (online suppl. table 2) chosen from the genomic sequence of the breakpoint clones.

Detection of CNVs was performed with an Illumina CytoS-NP-12 v2 microarray (Illumina Inc., San Diego, Calif., USA) according to the manufacturer's protocol. Array data were analyzed using GenomeStudio version 2011.1 and cnvPartition 3.2.0 (Illumina). A minimum cut-off value of 5 probes with a consistently aberrant signal was included in our criteria to ascertain a copy number change.

Sanger Sequencing and Targeted Next Generation Sequencing of Deafness Genes

Genomic DNA was extracted from whole blood using a standard salt extraction method. PCR was performed with an initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, and a final 10 min extension using M13 primers (Metabion, Munich, Germany) (online suppl. table 3), PCR buffer, nucleotide mixture and Fast Start Taq Polymerase (Roche Diagnostics). The 12 exons of *ATE1* and 26 coding exons of *SLC12A1* were Sanger sequenced with an ABI PRISM 377 (Applied Biosystems, Life Technologies, Carlsbad, Calif., USA). The sequence reaction was completed with 5× sequencing buffer and big dye terminator (Applied Biosystems). DNA sequence analysis was performed using NCBI BLAST. In the case of non-synonymous substitutions, PolyPhen-2 [Adzhubei et al., 2010], SIFT [Ng and Henikoff, 2001] and Alamut (Interactive Biosoftware, Rouen, France) were used to predict the impact of any identified amino acid substitution on the protein structure and function and the disease-causing potential.

Genomic DNA of the proband was submitted to Otagenetics Corporation (Norcross, Ga., USA) for exome capture targeting 130 known deafness genes and sequencing on a HiSeq2000 (Illumina). Paired-end reads of 90–100 bp were analyzed for quality, exome coverage and exome-wide SNP/InDels using the platform provided by DNAnexus (Mountain View, Calif., USA).

Fig. 1. Chromosome banding and FISH analysis of breakpoint regions. **a** G-banded karyotype of the index patient showing the apparently balanced derivative chromosomes. **b** G-banding pattern as well as corresponding ideograms showing the normal and derivative chromosomes resulting from the t(10;15)(q26.13;q21.1) translocation. Arrowheads indicate the translocation breakpoints.

c DAPI-stained (blue) metaphase spread of the index patient hybridized with digoxigenin-labeled (red) BAC RP11-78A18. The breakpoint-spanning BAC highlights the *ATE1* gene on the normal chromosome 10 as well as the 2 derivative chromosomes 10 and 15.

Zebrafish Whole Mount *in situ* Hybridization

Whole mount *in situ* hybridization was done to assess *ate1* and *slc12a1* expression in wild-type developing zebrafish (*Danio rerio*, wild-type TüAB strain) embryos. Antisense riboprobes for *ate1* and *slc12a1* were prepared using the pCRII-TOPO vector (Invitrogen, Darmstadt, Germany). A 646-bp fragment of *ate1* on zebrafish chr.Zv9_NA48 was isolated from the cDNA of whole embryos (26–28 hours post fertilization, hpf) using forward primer 5'-GGCCTCCTCCTCAAGTCTCT-3' and reverse primer 5'-GACACAAGAGGGCAGGAT-3' and was cloned into a pCRII-TOPO vector. For preparation of the *ate1* antisense riboprobe, pCRII-*ate1* was digested with *Bam*HI and transcribed with T7 RNA polymerase. Similarly, a 505-bp fragment of *slc12a1* on zebrafish chromosome 18 was isolated using forward primer 5'-TCTGCCTAAAGGGACTCTGC-3' and reverse primer 5'-CGAGGC-CAGAAAGAAGTTGG-3'. The fragment was cloned into a pCRII-TOPO vector. For preparation of the *slc12a1* antisense riboprobe, pCRII-*slc12a1* was digested with *Hind*III and transcribed with T7 RNA polymerase. *In situ* hybridization was performed as described in Winkler and Moon [2001]. Embryos were mounted in glycerol and photographed as whole mounts.

Results

Clinical Report

The patient is the first child of non-consanguineous German parents without a family history of hearing loss in childhood. His grandparents, parents and younger brother were healthy and normally hearing. Following an uneventful pregnancy, he was born at term with Apgar scores of 10 and 10 at 1 and 5 min, respectively. Birth

weight was 5,300 g (+4 SD), length was 62 cm (+4.5 SD) and occipitofrontal circumference 39 cm (+3.7 SD). The reasons for the macrosomia and macrocephaly remained unknown; in particular, there was no evidence for an overgrowth syndrome or for gestational diabetes. Newborn hearing screening (otoacoustic emission) was not performed. Development was normal and without notable diseases, and his overgrowth gradually decreased to normal. He could sit unassisted at age 6 months, spoke first words at 12 months and walked at 13 months. At the age of 6 years, his mother noticed hearing problems and bilateral sensorineural impairment was diagnosed. Pure tone audiometry showed a nearly symmetrical loss with normal hearing up to 1 kHz and hearing thresholds of 35 dB at 2 kHz, 40 dB at 4 kHz and 60 dB at 8 kHz, respectively. Speech and language had developed normally. His achievements in school and developmental testing (German version of the Developmental Test of Visual Perception) were ahead of age. The boy was fitted with hearing aids and his problems to understand at home and in school ceased. At the age of 7½ years, height was 120.3 cm (25th percentile) and weight 24.5 kg (25–50th percentile). Clinical investigation disclosed no abnormalities apart from a mild hyperopia that was also present in his younger brother. Thyroid function test, electrocardiogram and kidney ultrasound were normal, as was mutation analysis of common hearing loss genes including *GJB2* and *GJB6*.

Fig. 2. Graphic illustrations showing a translocation-mediated disruption of the *ATE1* and *SLC12A1* genes. The upper part shows a BAC (green) contig covering the *ATE1* breakpoint region on chromosome 10. Bars represent 20 kb. An expanded representation of the breakpoint including exon 12 with the end of the coding region and the 3' UTR region is shown in dark blue, and comprises a 10.1-kb interval. The bottom part shows a BAC contig covering the *SLC12A1* breakpoint on chromosome 15. An expanded representation of the breakpoint spanning introns 17–19 and exons 18 and 19 in light blue and dark blue, respectively, comprise an 8.7-kb interval.

Chromosome Banding Analysis

The patient was identified in a cytogenetic screen for disease-associated balanced chromosome rearrangements in >300 children with non-syndromic hearing impairment [Schneider et al., 2009]. GTG banding revealed an apparently balanced translocation between the long arms of chromosomes 10 and 15. At the cytogenetic level, the breakpoints were assigned to bands 10q26.11q26.13 and 15q21.1q21.2 (fig. 1a, b). Karyotyping of the parents and family (data not shown) revealed that the translocation had been inherited from the father and was also present in the proband's brother and paternal grandfather, all with normal hearing. The mother and the paternal grandmother had normal female karyotypes.

Positional Cloning of Translocation Breakpoints

Once the breakpoints were provisionally delineated, FISH experiments were used to refine the breakpoint location. Online supplementary table 1 lists the BAC clones that were selected with their corresponding mapping results. BAC RP11-78A18 (83 kb) hybridized to the normal chromosome 10 and both derivative chromosomes 10 and 15 (fig. 1c), as expected for a chromosome 10-breakpoint spanning clone. Similarly, we identified a break-

point-spanning BAC clone, RP11-154J22 (101 kb) on chromosome 15. Based on the FISH results, the proband's karyotype was refined to 46,XY,t(10;15)(q26.13;q21.1).

To further narrow down the breakpoint regions, we generated long-range PCR products from the breakpoint-spanning clones on chromosomes 10 and 15 (online suppl. table 2). The FISH mapping of individual long-range PCR products assigned breakpoints to a 10.1-kb interval (amplicon 6 of BAC RP11-78A18) on chromosome 10 localizing to exon 12 including the end of the coding sequence and the 3' UTR of the *ATE1* gene and an 8.7-kb interval (amplicon 2b of BAC154J22) on chromosome 15 between introns 17 and 19 of the *SLC12A1* gene. Breakpoint intervals are illustrated in figure 2.

Consistent with the UniGene database (<http://www.ncbi.nlm.nih.gov/unigene>), we found that *ATE1* but not *SLC12A1* is expressed in blood. Although we localized the translocation breakpoints to predicted regions within 2 genes, we were unable to successfully detect fusion gene transcripts in the proband (data not shown). However, because of the 3'–5' orientation of *ATE1* and the 5'–3' orientation of *SLC12A1* in the derivative chromosomes, the possibility of an *ATE1*-*SLC12A1* or *SLC12A1*-*ATE1* fusion transcript is unlikely due to the opposing transcriptional directions.

In addition to direct gene disruption, the translocation could indirectly affect the regulation of genes within a flanking one megabase region of *ATE1* and *SLC12A1*. There are several interesting candidate genes in these regions that, while not associated with a deafness phenotype, demonstrate expressed sequence tags in the human ear (online suppl. table 4).

Mutation Analyses

According to DECIPHER version 5.1 (<http://decipher.sanger.ac.uk>), both *ATE1* and *SLC12A1* are not thought to affect the phenotype by way of haploinsufficiency. In order to test whether gene disruption by translocation unmasks a recessive mutation in the second allele of our proband, we sequenced all 12 coding exons of *ATE1* (MIM 604103) and all 26 coding exons of *SLC12A1* (MIM 600839) and did not detect any pathogenic mutation. He was homozygous for 2 synonymous SNPs c.303A>G (rs10749435) and c.1236A>G (rs4237536) in *ATE1* and heterozygous for one non-synonymous benign SNP c.2873T>C, p.Val958Ala (rs1552311) in *SLC12A1*.

In addition, we performed a mutation screening in 180 *GJB2* mutation-negative children with non-syndromic hearing impairment. Fifteen to 20% had mild, the remaining cases moderate to profound hearing impairment. Al-

Table 1. Results for *ATE1* and *SLC12A1* mutation screening in 180 patients

Gene	Exon	Nucleotide change	Amino acid change	rdbSNP (build 13)	PolyPhen-2 prediction ^a	SIFT prediction ^b
<i>ATE1</i> (exons 1–12)	1	c.9C>T	p.Phe3Phe	rs79570924		
	1	c.88T>G	p.Ser30Ala		benign (0.000)	tolerated (0.83)
	3	c.198C>T	p.Val66Val			
	4	c.303A>G ^c	p.Ala101Ala	rs10749435		
	5	c.499C>T	p.Leu167Phe	rs148095496	benign (0.000)	tolerated (0.09)
	9	c.1125G>A	p.Ser375Ser	rs139300996		
	9	c.1140C>T	p.Gly380Gly	rs150860078		
	10	c.1208A>G	p.Tyr403Cys	rs148135505	probably damaging (1.000)	deleterious (0.00)
	10	c.1236A>G	p.Ser412Ser	rs4237536		
	11	c.1371A>C	p.Pro457Pro	rs35350755		
<i>SLC12A1</i> (coding exons 2–27)	7	c.828G>A	p.Val276Val	rs3825960		
	13	c.1539C>T	p.Val513Val			
	14	c.1614T>C	p.Tyr538Tyr	rs6493311		
	18	c.2067G>A	p.Gly689Gly	rs35783293		
	24	c.2873T>C	p.Val958Ala	rs1552311	benign (0.000)	tolerated (0.77)

^a PolyPhen-2 operates on a scale from 0 to 1.0, with 1.0 having the highest probability of being a damaging substitution.

^b SIFT values <0.05 predict substitutions that are deleterious, whereas values >0.05 predict tolerated substitutions.

^c The NCBI SNP database listed c.303G>A and not c.303A>G. This could be the result of a manual entry error.

together, the *ATE1* gene sequencing identified 7 synonymous, 2 benign non-synonymous and 1 probably damaging or deleterious base change (table 1). The potentially pathogenic c.1208A>G (p.Tyr403Cys) mutation was present in a heterozygous state in a single individual with severe hearing impairment in the lower frequencies that improves to normal hearing at higher frequencies (particularly at 6 and 8 kHz). She also suffers from recurrent bilateral tinnitus for at least 10 years. This mutation occurs in a highly conserved nucleotide and amino acid, up to Baker's yeast (considering 12 species) and has a large physicochemical difference existing between Tyr and Cys with a Grantham distance of 194 (Alamut version 2.0). This variation is also entered in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>) as rs148135505, with a MAF of 0.001 (1000 Genomes) and is in the Exome Variant Server database (<http://evs.gs.washington.edu>) with a similar MAF of 0.002 in the European population. The *SLC12A1* sequencing detected 4 synonymous and 1 non-synonymous variant (table 1).

Targeted Next Generation Sequencing of Deafness Genes and SNP Array Analysis

Because the translocation was segregating in both affected and unaffected family members and we did not find evidence for mutations in *ATE1* and *SLC12A1* underlying hearing impairment, we used targeted deafness gene enrichment sequencing (Otogenetics) to screen for mutations in 130 known deafness genes. The analysis strategy we employed disclosed only apparently non-pathogenic variants. Notably, one non-synonymous heterozygous change, c.1985G>A, p.Gly662Glu, in myosin IA (*MYO1A*, MIM 601478), a major contributor to autosomal dominant hearing loss (DFNA48) [Donaudy et al., 2003], was reported as possibly pathogenic by UniProt through the Alamut mutation report. However, because this variant is in a position of the *MYO1A* motor domain that is not evolutionarily conserved and also listed as a SNP (rs33962952), it is unlikely to cause hearing impairment. The fact that this SNP was found in the normally

Fig. 3. Whole-mount in situ hybridization of wild-type zebrafish embryos shows the expression pattern of *ate1* and *slc12a1* during embryogenesis. **a** 91-hpf lateral view depicting strong *ate1* expression in the fin bud (arrow) and heart (filled arrowhead) with minor expression in neuromasts (open arrowheads). **b** A polar view of the 15–16-hpf somite stage with *slc12a1* expression in the distal early pronephros (bracket).

hearing father and paternal grandfather of our proband also argues against a dominant causative mutation, although the formal possibility of reduced penetrance in unaffected family members cannot be excluded.

In some cases of apparently balanced chromosome rearrangements, microdeletions and/or duplications in the vicinity of the breakpoint regions [Yue et al., 2005] or elsewhere in the genome [Baptista et al., 2008] were found to be disease-causing. The SNP array analysis did not reveal any cryptic gain or loss of chromosome material in the proband (data not shown). There was no detectable difference in the breakpoint regions between affected and unaffected translocation carriers. The proband displayed a 4-Mb copy neutral loss of heterozygosity on chromosome 17 (50,329,974–54,383,237 bp; NCBI 37/hg19); however, this region does not contain any strong deafness candidate genes. Interestingly, when investigating known copy number variation in the breakpoint intervals in the database of genomic variants [Zhang et al., 2006], *ATE1* lacks genomic variation and *SLC12A1* has only one indel reported in 270 HapMap controls.

Expression of *ate1* and *slc12a1* during Zebrafish Development

According to UniGene, neither *ATE1* nor *SLC12A1* appears to be expressed in the human ear. To evaluate the possible functional role of *ATE1* and *SLC12A1* during ear development, the expression patterns were investigated in zebrafish embryos using *ate1* and *slc12a1* cDNA probes. Major expression of *ate1* was marked at 91 hpf in the heart and fin bud, with minor expression in the neuromasts (fig. 3a). Prominent expression of *slc12a1* was observed in the distal early pronephros in all stages beginning with the somite stage at 15–16 hpf, at 30 hpf and in 96 hpf (fig. 3b). As expected, no signal was visualized with the sense riboprobes.

Discussion

The proband inherited a balanced translocation t(10;15) from his phenotypically normal father; thus, it is unlikely that the translocation alone is by itself the cause of deafness. However, interplaying with other genetic and/or environmental factors, it could contribute to the events leading to hearing loss. Upon the identification of the 2 disrupted genes in the breakpoint regions (*ATE1* in 10q26.13 and *SLC12A1* in 15q21.2), we began a candidate gene approach involving bidirectional sequencing of all exons of each of the disrupted genes in the proband as well as a pool of 180 hearing impaired individuals.

To date, there are no disease-causing mutations or phenotypes associated with *ATE1* in humans that have been cataloged in databases such as HGMD (<http://www.biobase-international.com/product/hgmd>) and Swiss-Var (<http://swissvar.expasy.org>). Evidence from *Ate1*^{−/−} knockout mice suggests that this gene is important for cardiovascular development [Kwon et al., 2002]. We show strong expression of the zebrafish ortholog in the developing heart (and fin bud). To the best of our knowledge, neither the proband nor affected family members with the described translocation have heart defects. An interesting point for discussion is that there was weak expression of *ate1* observed in the neuromasts of zebrafish. Neuromasts are, in morphology and functionality, similar to hair cells of the ear. Consistent with the in silico prediction (DECIPHER) that *ATE1* is not sensitive to haploinsufficiency, heterozygous disruption of *ATE1* alone can be tolerated. Posttranslational arginylation by *ATE1* is an important biological regulator of protein properties and function. The tRNA-dependent addition of Arg onto proteins is essential for mammalian embryo-

genesis and tissue morphogenesis [Saha and Kashina, 2011]. In this light, it is tempting to speculate that arginylation of a potential ATE1-target protein, which is essential for normal hearing development, is altered by a second mutation that is present in the genome of the proband, but not in his healthy brother and father. Arginylation of hearing loss genes is not well investigated. However, differential arginylation of actin isoforms is well known [Zhang et al., 2010] and both beta and gamma actins have specific roles for auditory hair cells [Perrin et al., 2010].

In our cohort of hearing-impaired individuals, we found one potentially pathogenic mutation affecting a highly conserved nucleotide and amino acid (c.1208A>G, p.Tyr403Cys) in the *ATE1* gene in a heterozygous state. Together with our translocation patient, 2 of 180 studied individuals exhibit only one wild-type *ATE1* allele. However, we have not assessed the pathogenicity of the identified mutations on a functional level. The Exome Variant Server database contains 2 additional probably pathogenic frameshift mutations which are not in dbSNP v135. The c.427_430del4 deletion with a MAF of 0.004 in Europeans affects the protein by inducing a premature stop codon (p.Leu143Valfs*8). The c.93_94insC insertion (p.Ser32Glnfs*72) with a MAF of 0.04 is listed with a read depth of only 8 and, therefore, a sequencing artifact cannot be excluded.

Homozygous or compound heterozygous mutations in *SLC12A1* are associated with antenatal Bartter syndrome type I (MIM 601678), a life-threatening renal tubular disorder [Simon et al., 1996]. In this light, it is not unexpected that our zebrafish experiments showed strong *slc12a1* expression in the pronephros. None of the translocation carriers were reported to suffer from renal problems. However, it is noteworthy that 2 of 9 Costa Rican Bartter syndrome patients with a homozygous premature

stop mutation in the *SLC12A1* gene exhibited sensorineural hearing loss [Kurtz et al., 1997]. Considering that the incidence of Bartter syndrome (with at least 5 different types) is approximately 1 in 800,000–1,000,000 (<http://www.orpha.net>), the frequency of heterozygous *SLC12A1* mutation carriers is estimated to be approximately 1 in 1,000. The risk of our index patient for a child with Bartter syndrome is then approximately 1 in 4,000.

In conclusion, heterozygous disruption of *ATE1* and/or *SLC12A1* gene function is benign in nature and does not cause a haploinsufficient state. Additionally, from sequencing data, the proband lacks a second mutation in either of these genes. Since we eliminated the contribution of another known deafness gene by performing sequencing of 130 genes associated with deafness, we can argue in favor of the notion that the proband suffers from a polygenic or multifactorial form of deafness. On the other hand, we cannot exclude the possibility that the translocation and hearing impairment are coincidental or that our proband has a pathological mutation in a gene that is not yet associated with hearing loss. Collectively, our study emphasizes the difficulty of finding causative mutations in patients with non-syndromic hearing loss. Such particularly challenging patients may become targets for novel techniques such as whole exome or genome sequencing.

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