A TMEM43 autosomal dominant variant as the causative genetic mechanism for ANSD

August 2021—Auditory neuropathy spectrum disorder is a rare, progressive condition affecting a person’s ability to discriminate speech despite being able to respond to sound. The condition is often sporadic and diagnosed in patients with no family history of hearing loss, but it can be heritable. Hearing loss is categorized as conductive, which involves the middle ear; sensorineural, which involves the inner ear; or mixed, which is due to congenital, infectious, neoplastic, mechanical, aging, or environmental causes. In humans, sound is transmitted from the inner ear to the brain via the auditory nerve, which transmits nerve impulses created by the translation of sound to electrical current by the inner hair cells of the organ of Corti in the cochlea. Although the cause of auditory neuropathy spectrum disorder (ANSD) is unknown, dysfunction and disease affecting the sensorineural compartment, including defects potentially caused by certain hereditary gene mutations, have been proposed. The authors performed an unbiased genetic analysis of two families with members from five generations who were affected with ANSD. They found a nonsense variant in transmembrane protein 43 (TMEM43) p.(Arg372Ter) in the affected individuals. This variant was then functionally characterized in vitro and in an in vivo mouse disease model. The initial whole genome linkage analysis identified a putative locus at 3p251. Subsequent exome sequencing and population database filtering identified TMEM43 p.(Arg372Ter) as the likely candidate gene variant associated with the condition. Characterization of wild-type TMEM43 showed its expression in cochlear glia-like supporting (GLS) cells of the organ of Corti, which reside adjacent to hair cells and play a critical role in the development and maintenance of the auditory system. TMEM43 is localized in the plasma membrane of inner border cells and cell junctions of the inner sulcus cells. In protein interaction studies, it was found to directly interact with the connexin 26 and connexin 30 gap junction channels, which are involved in regulating intracellular K+ and maintaining pH homeostasis, supporting the role of TMEM43 in gap junction channel function in the cochlea. The pathogenic TMEM43 variant introduces a premature stop codon, resulting in truncation of the last 29 amino acids of the protein, including the fourth transmembrane domain and extracellular C-terminal domain. In genetically modified knock-in (KI) mice harboring the human TMEM43 p.(Arg372Ter) variant, auditory function, measured by auditory brain response, demonstrated progressive hearing loss beginning at five months of age. When compared to wild-type mice, the KI mice demonstrated a morphologic size reduction in GLS cells but not in their shape, arrangement, number of synaptic ribbons, or gross differences between stereocilia. Functional assays with whole-cell patch clamp of GLS cells derived from cochlear tissue demonstrated a significant reduction in passive conductance current in mice that were heterozygous (63 percent reduction) and homozygous (69 percent reduction). The reduced passive conductance current was restored in the heterozygote but not the homozygote tissue with a TMEM43 variant-specific shRNA to disrupt the knock-in allele. This supports the autosomal dominant effect of the TMEM43 p.(Arg372Ter) pathogenic variant. Identifying the cause of hearing loss is critical for appropriate clinical intervention. Hearing aids are appropriate for conductive hearing loss or moderate sensorineural hearing loss; surgical placement of cochlear implants is appropriate for patients with profound sensorineural hearing loss; and brain stem implants are used for patients with more central hearing loss. Based on the genetic pathophysiology detailed in this study, three ANSD subjects were treated with cochlear implants. Two of the subjects had a deafness duration of approximately 15 years, and one had a deafness duration of approximately 25 years. The ability to discriminate speech was restored with the cochlear implant in all three subjects, with restoration delayed in the subject who had been deaf longer. In summary, the authors have identified a novel deafness gene TMEM43 p.(Arg372Ter) variant that could be included in the genetic evaluation of people with hearing loss. Cochlear implants were efficacious in these individuals.


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Detection of tumor-derived DNA in circulating cell-free DNA in select pediatric cancers

The discovery that patients with solid tumors have tumor-derived DNA circulating in peripheral blood as cell-free DNA has led to the implementation of novel molecular tests using a blood sample (liquid biopsy) in lieu of traditional tissue biopsy. While the FDA approved the use of next-generation sequencing-based testing on liquid biopsies last year, it is not the standard of practice in many molecular diagnostic laboratories due to practical and technical hurdles. Most molecular
testing of solid tumors is focused on detecting genetic aberrations (somatic mutations, fusion genes, and copy number alterations) in the tumor using targeted next-generation sequencing gene panels, exome sequencing, low-coverage whole genome sequencing, and droplet digital PCR. These approaches are often inadequate for liquid biopsies due to such issues as low sensitivity and lack of readily detectable genetic aberrations. Consequently, there is an unmet need for new approaches to liquid biopsy analysis in pediatric tumors, particularly accounting for the low rate of recurrent genetic aberrations in most childhood cancers. A potential solution may involve analyzing epigenetic changes affecting chromatin structure and DNA methylation signature patterns that often yield a molecular fingerprint that distinguishes between noncancer and cancer and between different types of cancers. The authors conducted a study in which they performed a large integrated genetic and epigenetic analysis of circulating tumor DNA from pediatric cancer patients and demonstrated a number of clinically relevant technical and analytical advances. In the study, genetic analysis of cell-free DNA (cfDNA) from Ewing sarcomas (n = 95), other pediatric sarcomas (n = 31), and healthy controls (n = 22) included deep whole exome sequencing, EWS-Ets fusion oncogene quantification, and copy number alteration quantification. Epigenetic analysis of cfDNA included genomewide cfDNA fragmentation pattern analysis, a recently described method to detect naturally fragmented DNA reflective of chromatin structure, and DNA methylation profiling. Both provide signatures specific to the tumor of origin. The authors demonstrated that pediatric sarcomas have tumor-specific cfDNA fragmentation patterns that are similar to those previously reported for adult cancers. The patterns can be exploited to detect cancer in cfDNA from tumors with few, if any, recurrent genetic aberrations. The authors found chromosomal regional differences by cfDNA fragmentation patterns that corresponded with tumor-specific chromatin structure, providing a unique molecular fingerprint of the tumor. They developed a dedicated analysis method and software tool, named Liquorice, for fragmentation analysis of cfDNA in the context of predefined chromosomal regions of interest. The tool can be used to detect and quantify tumor-derived DNA independent of recurrent genetic aberrations. Comparison of genetic and epigenetic test results of cfDNA using multiple machine-learning classifiers demonstrated greater detection sensitivity with the DNA fragment-based approach than the current genetic-based approaches. Furthermore, the DNA fragment method outperformed genetic-based methods in distinguishing between different pediatric sarcomas. Detection of cfDNA through DNA fragment analysis may have prognostic value in pediatric sarcomas given that the detection sensitivity of the method and its correlation with the detection of tumor-derived DNA in the blood have been linked to poor clinical outcomes in pediatric cancers.


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