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A novel PIK3CD C896T mutation detected in bilateral sudden sensorineural hearing loss using next generation sequencing: An indication of primary immunodeficiency☆

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Abstract

Objective: To investigate immune-related genetic background in bilateral sudden sensorineural hearing loss (SSNHL).

Case report and methods: The case is a 45-year-old man presenting with a 7-year history of bilateral profound SSNHL. Blood biochemical testing demonstrated increased levels of total cholesterol (5.88 mmol/L). Tests for hepatitis B showed a positive antibody against the hepatitis B core antigen. Complement C3 was below the normal value, and complement C4 and IgG were in the lower range of normal values. CT images showed a normal inner ear and vestibular aqueduct but round window membranous ossification on both sides. A total number of 232 immune-associated genes were sequenced using the next generation sequencing technique.

Results: Mutations were detected in 5 genes, including the phosphoinositide 3-kinase catalytic subunit delta (PIK3CD), caspase recruitment domain-containing protein 9 (CARD9), complement factor H-related (CFHR2), immunoglobulin lambda-like polypeptide 1 Protein (IGLL1), and transmembrane channel-like gene family 8 (TMC8). In the PIK3CD gene, a C896T substitute in exon 7 was detected. This mutation causes primary immunodeficiency and is an autosomal dominant disease.

Conclusion: The PIK3CD C896T mutation responsible for primary immunodeficiency may contribute to the onset of bilateral SSNHL with subsequent rapid progression.

Keywords: Sudden sensorineural hearing loss; Immunology; Genetics; Next generation sequencing

1. Introduction

Sudden sensorineural hearing loss (SSNHL) has an acute onset and severely affects patient quality of life by limiting their ability to communicate with others (Stachler et al., 2012). The etiology of SSNHL is unclear, and viral infection, immune dysregulation and metabolic disorders may be involved in the development of the disease (Aimoni et al., 2010; Cadoni et al., 2002; Liao et al., 1992; Mayot et al., 1993; Passamonti

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et al., 2015; Pyykko and Zou, 2008; Veldman et al., 1993). The treatment for SSNHL remains controversial. In stark contrast to unilateral SSNHL, bilateral SSNHL is less common, but has specific distinguishing characteristics and is most often associated with toxic, autoimmune, neoplastic and vascular conditions. Patients with bilateral SSNHL have more severe (often profound) hearing loss, poorer recovery and a 35% mortality rate (Oh et al., 2007; Sara et al., 2014; Xenellis et al., 2007). There is an urgent need to uncover the genetic background of patients with bilateral SSNHL in order to develop more efficient prophylactic and therapeutic strategies.

Here, we report a case of bilateral SSNHL with rapid profound hearing loss following a common cold, aiming at exploring the status of immune-related genes. All 232 candidate genes involved in immune activity, selected according to Human Phenotype Ontology (http://human-phenotype-ontology.github.io/), were sequenced using next generation sequencing, a high-throughput method to foster novel discovery in biomedical research. The significance of the results is discussed with reference to recent publications.

2. Case presentation

2.1. Clinical data

A 45-year-old man presented with a 7-year history of bilateral SSNHL. During a night in early January 2008, he slept in a cold room without covering and experienced a fever the next morning with a mild occipital headache at noon that became severe at 4 pm. He took an unknown medicine for fever in the evening. On the third night, he took a traditional Chinese medicine for common cold (name unknown) for continuing fever and headache and slept until noon. Upon waking up, he became deaf in both ears, accompanied by unsteadiness. There was no tinnitus, ear fullness, nausea, or vomiting. He did not receive formal treatment. He was seen by authors on June 18, 2015 for treatment of his deafness. Neither air nor bone conduction pure tone audiometry detected any response at 0.25–8 kHz at the maximum output (Fig. 1). He was admitted on November 4, 2015 for cochlear implantation. The patient suffered from hepatitis B in 1988.

Pre-operative routine blood tests showed increased levels of hematocrit (52.8%), lymphocytes (43.4%) and hemoglobin (170 g/L), and decreased neutrophils (45.4%). Routine urine and stool tests were normal. Coagulation test was normal. Blood biochemical tests demonstrated increased levels of blood urea nitrogen (7.2 mmol/L), total cholesterol (5.88 mmol/L), high density lipoprotein (1.92 mmol/L), low density lipoprotein (3.76 mmol/L) and apolipoprotein A1 (2.12 g/L). Hepatitis B tests were positive for anti-core antigen antibody, and negative for surface antigen, E-antigen, anti-surface antigen IgG antibodies or anti-E-antigen IgM antibody. Antibodies against hepatitis C virus, human immunodeficiency virus or syphilis were negative. Auditory brainstem responses could not be induced in either ear to click stimulation at 80 dB nHL. Neither ocular vestibular-evoked myogenic potentials (oVEMPs) nor cervical vestibular-evoked myogenic potentials (cVEMPs) were elicited to 500 Hz tone burst stimulation at 97 dB nHL. CT images showed normal inner ear and vestibular aqueduct but round window membranous ossification on both sides (Fig. 2). Heavy T2-weighted MRI showed normal fluid patterns in the inner ear on both sides (Fig. 3). He received a cochlear implant (CS-10A, Zhejiang Nurotron Biotechnology Co., Ltd., Hangzhou, China) in right ear on November 9, 2015. Right side round wind membrane ossification was substantiated during surgery. The patient was not satisfied with the hearing outcomes at 4 months post-implantation, citing narrow dynamic range and broadness of the response, as well as monotonal sounds, which made understanding speech difficult, especially in noise. EDTA treated blood was taken on November 10, 2015 for gene sequencing. Pursuit tracking, optokinetic nystagmus, sera immunoglobulin levels, C-reactive protein, and complements C3 and C4 tests were completed on March 15, 2016.

2.2. Gene sequencing

A total of 232 immune-associated genes were sequenced using an Illumina HiSeq 2000 Sequencer (Illumina, California, USA) (Supporting material-Table 1). After DNA extraction from peripheral white blood cells, the sequenced sample was prepared according to the Illumina protocol. Briefly, 3 μg of genomic DNA was fragmented by nebulization (Covaris S2 system, Thermo Fisher Scientific Inc., Waltham, USA), and the fragmented DNA was repaired. An 'A' was ligated to the 3′ end, Illumina adapters were then ligated to the fragments. The sample was size selected for a 350–400 base pair product, which was PCR amplified (for primer information see Supporting material-Table 2A), and the final product was validated using the Agilent Bioanalyzer. The amplified DNA was captured with a target region related gene enrichment system (MyGenostics, MD, USA) based on previously described technologies (Huang et al., 2013). The capture experiment was conducted according to the manufacturer's protocol. In brief, a 1 μg DNA library was mixed with buffer BL and the GenCap target region probe (MyGenostics, MD, USA) and then heated at 95 °C for 7 min and 65 °C for 2 min on a PCR machine. Next, 23 μl of the 65 °C pre-warmed buffer HY (MyGenostics, MD, USA) was added to the mixture, which was held at 65 °C with PCR lid heat on for 22 h for hybridization. Fifty microliters of MyOne beads (Life Technology) were washed in 500 μl 1× binding buffer 3 times and resuspended in 80 μl 1× binding buffer. We added 64 μl 2× binding buffer to the hybrid mixture and transferred this to a tube with 80 μl MyOne beads. The mixture was rotated for 1 h on a rotator. The beads were then washed with WB1 buffer at room temperature for 15 min once and with WB3 buffer at 65 °C for 15 min three times. The bound DNA was then eluted with elution buffer. The eluted DNA was finally amplified for 15 cycles using the following program (for primer information see Supporting material-Table 2B): 98 °C for 30 s (1 cycle), 98 °C for 25 s, 65 °C for 30 s, 72 °C for 30 s (15 cycles) and then 72 °C for 5 min (1 cycle). The PCR product was purified