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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

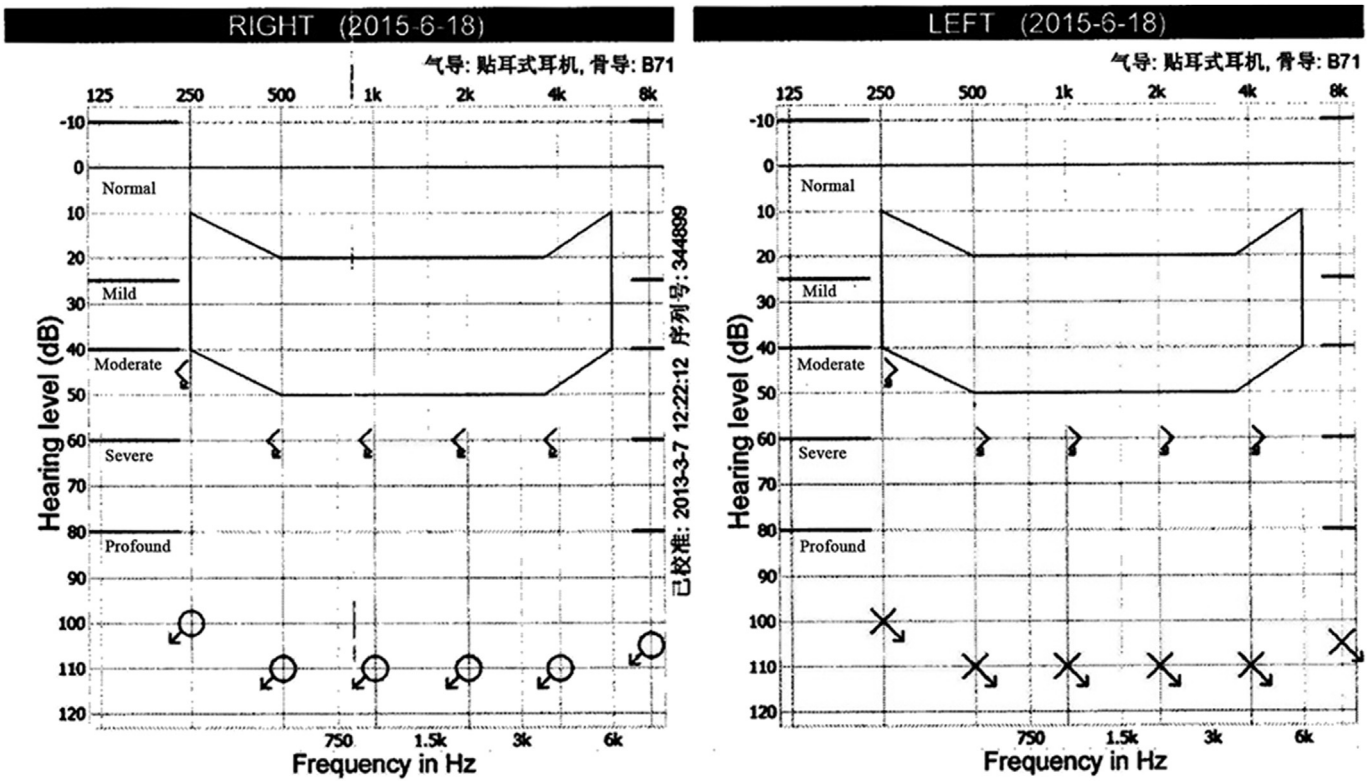

Fig. 1. Audiogram of a patient with bilateral sudden sensorineural hearing loss. Profound sensorineural hearing loss was detected in both ears.

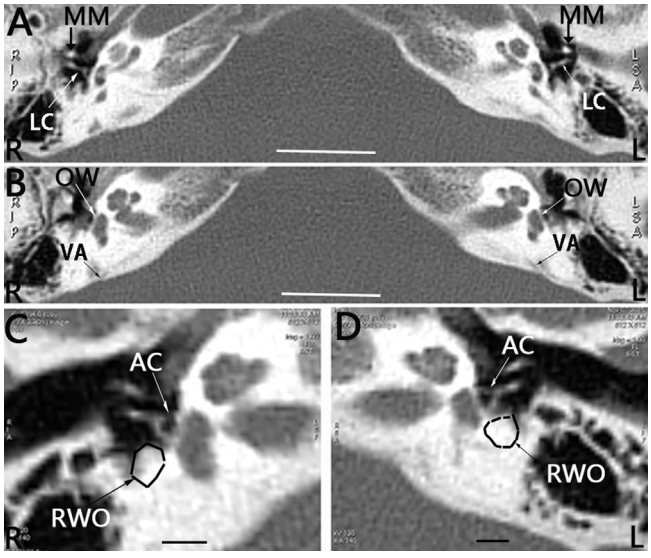

Fig. 2. CT image of the inner ear in a patient with bilateral sudden sensorineural hearing loss. The middle ear (A) and inner ear including the vestibular aqueduct (VA) are (B) normally developed. The bright signal in area of round window membrane (black frame) indicates round window membrane ossification (RWO) in both ears (C). AC: anterior crus of the stapes; LC: long crus of the incus; MM: manubrium of malleus; OW: oval window. Scale bars = 10 mm in A and B, and 5 mm in C and D.

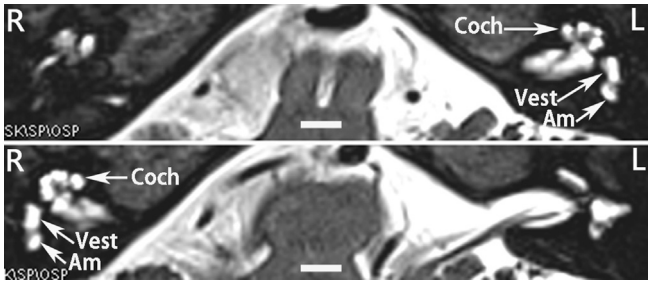

Fig. 3. Heavy T2-weighted MRI of the inner ear in a patient with bilateral sensorineural hearing loss. All compartments of the inner ear on both sides are filled with fluids. Am: ampulla; Coch: cochlea; Vest: vestibule. Scale bars = 5 mm.

using SPRI beads (Beckman Coulter Inc., California, USA) according to the manufacturer's protocol. The enrichment libraries were sequenced on an Illumina HiSeq 2000 sequencer for 100 bp paired reads. The data were analyzed as follows. Reference genome: The reads were mapped against UCSC hg19 (http://genome.ucsc.edu/). Read mapping: The reads were mapped by SOAPaligner (http://soap.genomics.org.cn/soapaligner.html) and Burrows-Wheeler Aligner (BWA) (http://bio-bwa.sourceforge.net/bwa.shtml). Variant detection: The SNPs and indels were detected by GATK and SOAPsnp (http://soap.genomics.org.cn/soapsnp.html). Variant database: dbSNP & 1000G. The mutated genes were validated using first generation sequencing with an ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific Inc., Waltham, USA) (for primer information see Supporting material-Table 2C).

As a result, mutations were detected in 5 genes, including 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) (Table 1). For the PIK3CD gene, a c.C896T substitute in exon 7 was detected. This mutation causes primary immunodeficiency, which is an autosomal dominant disease (Angulo et al., 2013; Lucas et al., 2014). A heterozygous c.A586G substitution in exon 4 of the caspase recruitment domain-containing protein 9 (CARD9) gene was found. Deficiency of the gene is a recently described autosomal-recessive primary immunodeficiency marked by susceptibility to candidiasis (Gavino et al., 2014). A deletion of c.655−656 in exon 5 of the complement factor H related protein 2 (CFHR2) gene was found, which may be involved in autosomal-recessive neovascular diseases (Kubista et al., 2011; Sethi et al., 2011; Zhang et al., 2008). A c.T485A substitute in exon 3 of the immunoglobulin lambda-like polypeptide 1 (IGLL1) gene was found. Mutations in this gene can result in an autosomal recessive disease of B cell deficiency and agammaglobulinemia (Khalili et al., 2014). A c.G1967A substitute in exon 16 of the transmembrane channel-like protein 8 (TMC8) gene was also revealed in the patient. Homozygous, invalidating mutations in either the TMC8 or TMC6 gene are found in approximately 75% of epidermodysplasia verruciformis patients and confer susceptibility to the disease (Orth, 2006; Ramoz et al., 1999, 2002). Most patients demonstrate autosomal recessive patterns of inheritance, although some exhibit X-linked recessive or autosomal dominant inheritance (Yoshida et al., 2014).

Pursuit tracking and optokinetic nystagmus tests showed normal results, which did not support a central nerve system lesion. Complement C3 was below the normal value, and complement C4 and IgG were in the lower range of normal values.

3. Discussion

In the present report, we presented results of genetic studies using next generation sequencing in a patient with bilateral SSNHL that revealed a novel mutation in the phosphoinositide 3-kinase catalytic subunit delta (PIK3CD) gene leading to primary immunodeficiency (Angulo et al., 2013; Lucas et al., 2014) together with mutations in another 4 genes associated with autosomal-recessive primary immunodeficiency. Although the other mutations were heterozygous in immune-related genes with autosomal recessive inheritance, a carrier with a heterozygous mutation of an autosomal recessive gene has an increased health risk. Ataxia-telangiectasia is an autosomal recessive neurodegenerative disorder with immunodeficiency and an increased risk of developing cancer caused by mutations in the ataxia-telangiectasia mutated gene. Female carriers of the ataxia-telangiectasia mutation have an increased risk of breast cancer (van Os et al., 2015). In the present case, there might be a combined effect from mutations of the CARD9, CFHR2, IGLL1, and TMC8 genes as they are all associated with autosomal recessive diseases. Low levels of complements C3, C4 and IgG in the patient support the phenotype of immunodeficiency.

The present case of bilateral SSNHL with primary immunodeficiency is different from previous reports of an association with an autoimmune reaction (Sara et al., 2014). It is possible that the immunodeficiency status of the patient reduced the defending activity against the virus propagation that caused severe impairment in the inner ear. In addition to the low level of complement C3, the patient has a deletion of 655−656 in exon 5 of the complement factor H related protein 2 (CFHR2) gene. It was reported that complement factor H (CFH) polymorphism was significantly related to SSNHL (Nishio et al., 2012). CFH is a glycoprotein that plays a critical role in the regulation of the complement system through the assembly and decay of the alternative pathway C3 proconvertase and C3 convertase (Bettoni et al., 2016). The level of serum CFH correlates with the efficacy of opsonophagocytic killing of pneumococci (van der Maten et al., 2016). Viral and bacterial infections were reportedly involved in SSNHL (Cassilde et al., 2014; Dunne et al., 2004; Garcia Berrocal et al., 2000; Pyykko and Zou, 2008). The increased level of total cholesterol in the present case may also influence hearing prognosis (Quaranta et al., 2015). The poor outcome of cochlear implantation in the patient might result from severe degeneration of spiral ganglion cells during the infection (Seyyedi et al., 2014). An eABR test that estimates the

Table 1
Mutations detected in the immune-related genes using next generation sequencing and the resulting phenotypes.

Genes	MuLoc	NoTra	Exons	NuCh	AACh	Hom/Het	FreNor	Inh	Disease/phenotype
PIK3CD	chr1-9777132	005026	exon7	c.C896T	P299L	Het	0.0002	AD#	PID 14
CARD9	chr9-139265334	052813	exon4	c.A586G	K196E	Het	–	AR&	IFD
CFHR2	chr1-196928052 196928053	005666	exon5	c.655-656del	I219fs	Het	0.0020	AR¤	DCFHR
IGLL1	chr22-23915610	020070	exon3	c.T485A	M162K	Het	0.0383	AR¥	CAG
TMC8	chr17-76136979	152468	exon16	c.G1967A	S656N	Het	–	AR£	EDV

Supporting references as following: # Angulo et al., 2013; Lucas et al., 2014. & Gavino et al., 2014. ¤ Kubista et al., 2011; Sethi et al., 2011; Zhang et al., 2008. ¥ Khalili et al., 2014. £ Yoshida et al., 2014. AD: autosome dominant; AR: autosome recessive; BuLoc: nucleotide changes; CAG: congenital agammaglobulinemia; DCFHR: deficiency in complement factor H-related protein; del: deletion; EDV: epidermodysplasia verruciformis; FreNor: mutation frequency in normal individuals; Het: heterozygote; Hom: homozygote; IFD: invasive fungal disease; Inh: inheritance; NoTra: number of transcripts; PID: primary immunodeficiency.

responses of spiral ganglion cells should have been included in pre-cochlear implantation evaluation and can help predict CI outcomes, which however is not done as a routine test in the clinic yet.

The patient did not provide a history of being susceptible to other infections. It is recently reported that PI3KD is a key regulator of $CD8^+$ T cell responses that integrates extrinsic cues, including those from other responding cells, to determine the collective behavior of $CD8^+$ T cell populations responding to infection. The authors observed that inactivation of PI3KD resulted in enhanced bacterial elimination by the innate immune system. However, the magnitudes of the primary and secondary $CD8^+$ T cell responses were reduced. Moreover, PI3KD activity was required for $CD8^+$ T cells to provide help to other responding $CD8^+$ cells (Pearce et al., 2015). We suspect that the immune system of the patient has sufficient reaction to combat mild infection.

In conclusion, the current case demonstrates that a PIK3CD C896T mutation responsible for primary immunodeficiency may contribute to the onset of bilateral SSNHL with a rapid progression.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joto.2016.06.001.

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