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Identification of Two Cases of Ciliopathy-Associated Diabetes and Their Mutation Analysis Using Whole Exome Sequencing

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Background: Alström syndrome and Bardet-Biedl syndrome are autosomal recessively inherited ciliopathies with common characteristics of obesity, diabetes, and blindness. Alström syndrome is caused by a mutation in the *ALMS1* gene, and Bardet-Biedl syndrome is caused by mutations in *BBS1-16* genes. Herein we report genetically confirmed cases of Alström syndrome and Bardet-Biedl syndrome in Korea using whole exome sequencing.
Methods: Exome capture was done using SureSelect Human All Exon Kit V4+UTRs (Agilent Technologies). HiSeq2000 system (Illumina) was used for massive parallel sequencing. Sanger sequencing was used for genotype confirmation and familial cosegregation analysis.
Results: A 21-year old Korean woman was clinically diagnosed with Alström syndrome. She had diabetes, blindness, obesity, severe insulin resistance, and hearing loss. Whole exome sequencing revealed a nonsense mutation in exon 10 of *ALMS1* (c.8776C>T, p.R2926X) and a seven base-pair deletion resulting in frameshift mutation in exon 8 (c.6410_6416del, p.2137_2139del). A 24-year-old Korean man had Bardet-Biedl syndrome with diabetes, blindness, obesity, and a history of polydactyly. Whole exome sequencing revealed a nonsynonymous mutation in exon 11 of the *BBS1* gene (c.1061A>G, p.E354G) and mutation at the normal splicing recognition site of exon 7 of the *BBS1* gene (c.519-1G>T).
Conclusion: We found novel compound heterozygous mutations of Alström syndrome and Bardet-Biedl syndrome using whole exome sequencing. The whole exome sequencing successfully identified novel genetic variants of ciliopathy-associated diabetes.

Keywords: *ALMS1*; Alstrom syndrome; Bardet-Biedl syndrome; *BBS1*; Ciliopathy; Diabetes mellitus; Next generation sequencing; Sanger sequencing; Whole exome sequencing

INTRODUCTION

Genetic mutations in ciliary proteins result in intracellular and intercellular signaling defects and consequently lead to multi-organ developmental disorders [1]. Ciliopathies share clinical features such as mental retardation, cystic kidney disease, retinal defects, polydactyly, obesity, and diabetes [2]. Alström syndrome and Bardet-Biedl syndrome are ciliopathies that involve

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multiorgan systems and share common clinical features of blindness due to retinal defect, early onset obesity and diabetes [3,4]. Both syndromes are diagnosed based on clinical findings and are confirmed by genetic testing [5,6]. Until recently, both syndromes were confirmed with Sanger sequencing in experienced laboratories [7,8]. However, it is now possible to determine the whole exome sequence variation using a next generation massive parallel sequencing technique [9]. In this study, we describe Korean patients with Alström syndrome and Bardet-Biedl syndrome who were genetically confirmed using whole exome sequencing and provide information that might lead to possible translational implications.

METHODS

After acquiring written informed consent from patients and family members, we sequenced whole exomes of peripheral blood DNA from affected probands. The SureSelect Human All Exon Kit V4+UTRs (Agilent Technologies, Santa Clara, CA, USA) was used for whole exome capture and HiSeq 2000 Sequencing System (Illumina Inc., San Diego, CA, USA) was used for massive parallel sequencing. The sequence reads were mapped against human genome 19 using Burrows-Wheeler Alignment software [10]. Sequence variation including single nucleotide polymorphisms and insertion/deletions were detected by The Genome Analysis Toolkit (GATK; Broad Institute, Cambridge, MA, USA) software [11]. Sanger sequencing was performed on DNA from the probands for genotype confirmation and the family members for familial cosegregation analysis.

RESULTS

Case 1: Alström syndrome

A 21-year-old Korean woman had diabetes since age 13, poorly controlled glucose level with obesity (body mass index [BMI], 27.9 kg/m^2), severe insulin resistance (total daily requirement of insulin, 190 IU/day; homeostatic model assessment for insulin resistance [HOMA-IR] index, 62.8; HOMA-β, 113.7), and acanthosis nigricans. She had nystagmus and photophobia when she was 1 year old, which led to childhood blindness. She also presented with mild sensorineural hearing loss, nonalcoholic steatohepatitis, renal dysfunction, and hypertension. Additionally, she had secondary amenorrhea caused by polycystic ovary syndrome since age 12. Her cardiac function was normal and did not have cognitive dysfunction or general intelligence impairment during her developmental period.

Whole exome sequencing revealed the patient to have a compound heterozygous mutation in the *ALMS1* gene. The first mutation was a seven base pair deletion resulting in a frame shift that introduced a new stop codon at chr2: 73,680,067 (National Center for Biotechnology Information build, NCBI build 37) in exon 8 of the *ALMS1* gene (c.6410_6416del, p.2137_2139del). The second mutation was a stop codon in exon 10 of the *ALMS1* gene (c.8776C>T, p.R2926X) at chr2: 73,717,865 (NCBI build 37). As there was no consanguineous relationship, other family members did not showed features of Alström syndrome. We confirmed these mutations by Sanger sequencing and found that the c.8776C>T mutation was maternally inherited (Fig. 1A). The seven base pair deletion might have been either inherited from the patient's father or newly introduced as a *de novo* mutation. Her father passed away several years ago, and we were not able to obtain his DNA.

Case 2: Bardet-Beidl syndrome

A 24-year-old Korean man was diagnosed with diabetes at age 19. He had polyphagia with severe obesity (BMI, 40.4 kg/m^2). His blood glucose level was well-controlled with glucagon-like peptide-1 (GLP-1) agonist combined with oral antihyperglycemic agent. With a GLP-1 agonist, his glycosylated hemoglobin decreased from 11.8% to 6.9% within 8 months. He had retinitis pigmentosa resulting in blindness since age 10. He had polydactyly of both hands and a personality disorder. We clinically diagnosed Bardet-Biedl syndrome. We investigated variants in *BBS1* to *BBS16*, using whole exome sequencing. There were no homozygous autosomal recessive variants in these genes. Whole exome sequencing revealed a mutation at the normal splicing recognition site of exon 7 of the *BBS1* gene (c.519-1G>T) at chr11: 66,283,331 (NCBI build 37). The splice site score for this variant was –6.4, which implies that it is functionally detrimental (http://www.fruitfly.org/seq_tools/splice.html) [4]. A second mutation was a novel nonsynonymous mutation in exon 11 of the *BBS1* gene (c.1061A>G, p.E354G) at chr11: 66,291,304 (NCBI build 37). The PolyPhen-2 score of the variant was 0.99 and predicted to be probably damaging (http://genetics.bwh.harvard.edu/pph/) [12,13]. Consanguineous mating was not involved in the patient's family. No family member had the typical phenotype of Bardet-Biedl syndrome. Sanger sequencing revealed that the splice site variant, c.519-1G>T, was maternally inherited and the nonsynonymous mutation,

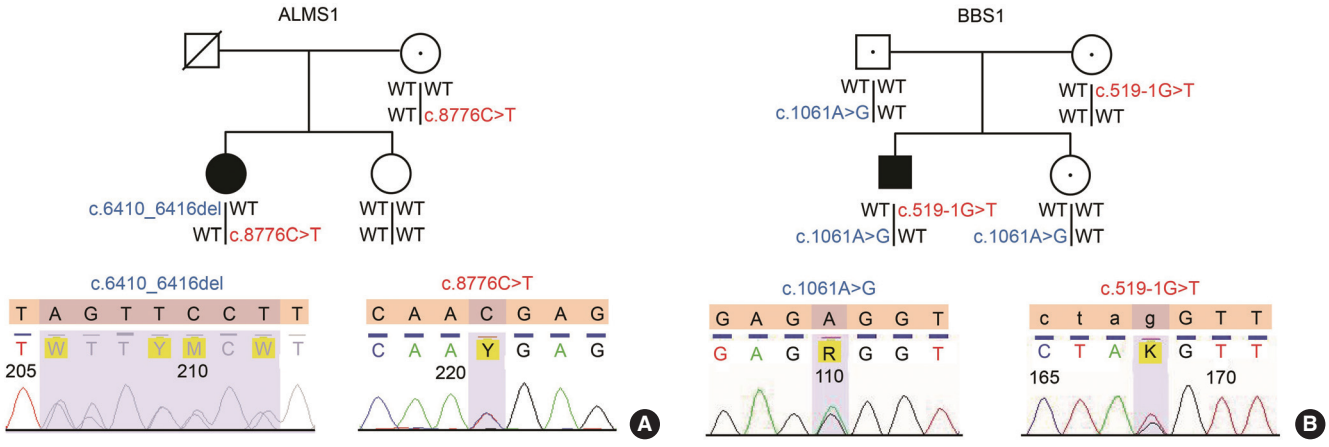

Fig. 1. Pedigrees of Alström syndrome and Bardet-Biedl syndrome patients. (A) The Alström syndrome patient had compound heterozygous mutations in the *ALMS1* gene. A mutation in exon 10 of the *ALMS1* gene (c.8776C>T, p.R2926X), which introduced a stop codon, was maternally inherited, and a seven base pair deletion in exon 8 of *ALMS1* gene mutation (c.6410_6416del, p.2137_2139del) might have been inherited from the patient's father or newly introduced as a *de novo* mutation. (B) The Bardet-Biedl syndrome patient also had a compound heterozygous mutation. A mutation at a normal splicing recognition site of exon 7 on the *BBS1* gene (c.519-1G>T) was maternally inherited, and a novel nonsynonymous mutation in exon 11 of *BBS1* gene (c.1061A>G, p.E354G) was paternally inherited. Circles, females; squares, males; filled symbols indicate affected members; empty symbols indicate healthy family members; symbols with dots in the center indicate obligate carriers; slashed symbols indicate those who had previously died. The DNA sequence electropherograms are shown for the reference (top) and subject (bottom). The sequences were analyzed using Variant Reporter v.1.1 (Applied Biosystems) with IUPAC (The International Union of Pure and Applied Chemistry) codes. WT, wild type.

c.1061A>G, was paternally inherited (Fig. 1B). In other words, the patient had a compound heterozygous mutation in *BBS1*.

DISCUSSION

Alström syndrome (OMIM 203800) is a rare autosomal recessive ciliopathy caused by *ALMS1* gene mutation. The prevalence of Alström syndrome is less than 1:1,000,000. The key features are childhood obesity, early-onset diabetes (70% by age 20), blindness from congenital retinal dystrophy, and sensorineural hearing loss [3]. It is reported that exons 8, 10, and 16 of the *ALMS1* gene are mutational hot spots. Patients with mutations in exon 8 seem to have less renal involvement than those with exon 16 mutations [14]. In this paper, we identified a compound heterozygous mutation in exon 8 and 10 of the *ALMS1* gene, the patient also showed mild to moderate renal insufficiency. Before the advent of the whole exome sequencing technology, Sanger sequencing was used for genetic diagnosis of Alström syndrome [5]. As the *ALMS1* gene consists of 23 exons and the size of the transcript is 12,922 base-pairs, it would require a large number of polymerase chain reaction (PCR) primers. In addition, we could not exclude the possibility of phenocopy or locus heterogeneity. Therefore, we opted for whole exome sequencing rather than Sanger sequencing. We used Sanger sequencing for both genotype validation and segregation analysis and confirmed the causality of the genetic variant. In previous reports, only one case of Alström syndrome has been reported in a Korean but without genetic confirmation [15]. This is the first genetically confirmed case of Alström syndrome using whole exome sequencing.

Bardet-Biedl syndrome (OMIM 209900) is also a rare autosomal recessive ciliopathy caused by at least 16 genes, including *BBS1* to *BBS16*. The estimated prevalence is 1:160,000 in northern European populations. Bardet-Biedl syndrome is characterized by retinal dystrophy (>90%), diabetes (<25%), truncal obesity (72%), post-axial polydactyly, cognitive impairment, male hypogonadotrophic hypogonadism, and renal abnormalities [4]. Bardet-Biedl syndrome is differentiated from Altsröm syndrome by the presence of polydactyly, cognitive impairment, less severe hearing loss and delayed onset type 2 diabetes mellitus [3,4]. Genotype-phenotype correlations are poor, even though one study suggests a milder phenotype-associated with the common mutation found in *BBS1* [16]. In this paper, the subject had a compound heterozygous mutation in the *BBS1*

gene and had retinitis pigmentosa. Particularly, he had a good response to the GLP-1 agonist. Until recently, one case of Bardet-Biedl syndrome with *BBS10* gene mutation was reported in Korea with genetic confirmation by Sanger sequencing methods [17].

The mechanism of diabetes associated with ciliopathies is not fully understood. The most important mechanism of ciliary dysfunction associated diabetes is considered to be insulin resistance. In both patients, there were distinct clinical characteristics of insulin resistance with early onset obesity and hyperphagia. However, further studies are required to understand the detailed molecular mechanism of diabetes with ciliopathies.

Whole exome sequencing is an attractive alternative to Sanger sequencing as it does not require predesigned specific primer sets for PCR. In addition, the cost of whole exome sequencing is expected to decrease below that of the Sanger sequencing in cases where the gene to be sequenced is of a certain size or larger. Recently, the United States Food and Drug Administration approved using the next generation sequencer, Illumina's MiSeqDx for clinical application and to be marketed [18]. The pitfall of whole exome sequencing is that it does not evenly cover the whole exome and some parts of the genome might be omitted from the analysis. There could be sequencing error that could result in either false positive and false negative findings. Our study highlights a successful case of confirming novel genetic variants in a rare recessive disorder.

In summary, we report two Korean cases of ciliopathy-associated diabetes, Alström syndrome and Bardet-Biedl syndrome. We found novel compound heterozygous mutations of Alström syndrome and Bardet-Biedl syndrome using whole exome sequencing. Whole exome sequencing could be used for genetic diagnosis of ciliopathy-associated diabetes. Further translational research would be helpful for understanding functional mechanism of these variants.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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