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Cochlear implantation for hearing loss due to an A8296G mitochondrial DNA mutation

Yujiro Hoshi, Akinori Kashio*, Erika Ogata, Yusuke Akamatsu, Tatsuya Yamasoba

Department of Otolaryngology and Head and Neck Surgery, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-8655, Japan

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ABSTRACT

Objective: To characterize the clinical findings in a patient with hearing loss harboring an A8296G mitochondrial DNA mutation and the outcome of cochlear implantation.
Patients: A case report of a patient with hearing loss caused by an A8296G mitochondrial DNA mutation.
Intervention: The patient underwent cochlear implantation (CI).
Main outcome and results: Bilateral moderate to severe hearing loss was found at high school age and progressed to severe hearing loss bilaterally at the age of 22. The patient's low-tone hearing was relatively well preserved compared to high frequency, although it eventually declined. Speech perception in silence and at S/N10 improved to 100% and 92% for sentences, respectively, 3 years after CI.
Conclusions: We detailed the case of a patient with hearing loss due to an A8296G mitochondrial DNA mutation. Bilateral progressive hearing loss starting from high frequency was observed. Speech discrimination after CI was very good, indicating that a patient with this mutation is a good candidate for CI.

Introduction

Mitochondrial diseases are disorders caused by impairment of the mitochondrial respiratory chain. Organs that rely on aerobic energy production, such as the visual and auditory pathways, heart, central nervous system, and skeletal muscle, are primarily affected [1]. Hearing impairment is reported to affect more than half of patients with these diseases. Pathological mutations of the mitochondrial DNA (mtDNA) have been commonly found at the transfer RNAs (tRNAs). To date, more than 90 point mutations in 21 of the 22 mitochondrial tRNA genes have been reported [2,3]. Most of these mutations result in a decreased rate of mitochondrial protein synthesis, causing a deficiency in the energy metabolism of the cell [4]. Approximately 50 mutations of the tRNA genes have been associated with deafness [5]. The A8296G substitution in tRNA(Lys) was reported to cause hearing loss, although detailed findings and the clinical course of the hearing loss was not described. Herein we present the case of a patient with an A8296G mitochondrial DNA mutation who had progressive sensorineural hearing loss (SNHL) and underwent cochlear implantation (CI).

Case presentation

The patient was a Japanese woman who was born without any perinatal abnormalities. Neither she nor her family had a reported history of otitis media or diabetes. She was first diagnosed with bilateral hearing loss of approximately 70 dB HL during a medical examination at her high school. She started using hearing aids at age 15, but her hearing level gradually declined. At 20 years of age, her hearing thresholds had increased to 90 dB HL. She did not experience any benefit in speech discrimination while using hearing aids and stopped wearing them. She presented at our department at 21 years of age.

The patient's unaided average threshold was 91.3 dB HL in the right ear and 98.8 dB HL in the left ear, with some residual low-frequency hearing bilaterally (Fig. 1). Her phoneme speech discrimination score was 15% on the right and 0% on the left. She started using hearing aids again, but improvement in her speech discrimination was limited. Her hearing loss progressed steadily at all frequencies, and 10 months after her first visit to our department, the average threshold had declined to 105 dB HL on the right and 95 dB HL on the left (Fig. 2). No abnormality was observed in the temporal bone or brain on high-resolution computed tomography (HRCT) or magnetic resonance imaging (MRI). At 22 years of age, the patient underwent left CI surgery. A CI522 implant (Cochlear, Melbourne, Australia) was inserted and complete electrode insertion was achieved. An electrically evoked compound action potential could be elicited in all electrodes. During the postoperative follow-up, we conducted a gene-targeted sequence

* Corresponding author.
E-mail address: kashioa-tky@umin.ac.jp (A. Kashio).

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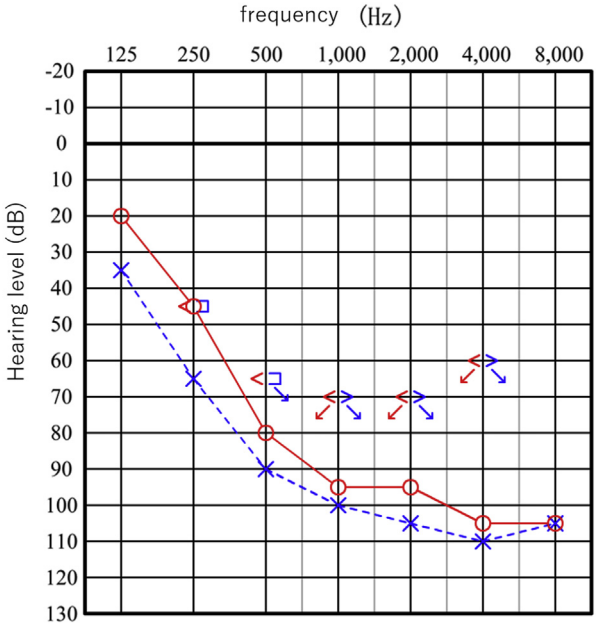

Fig. 1. Audiograms at the first visit to our department.

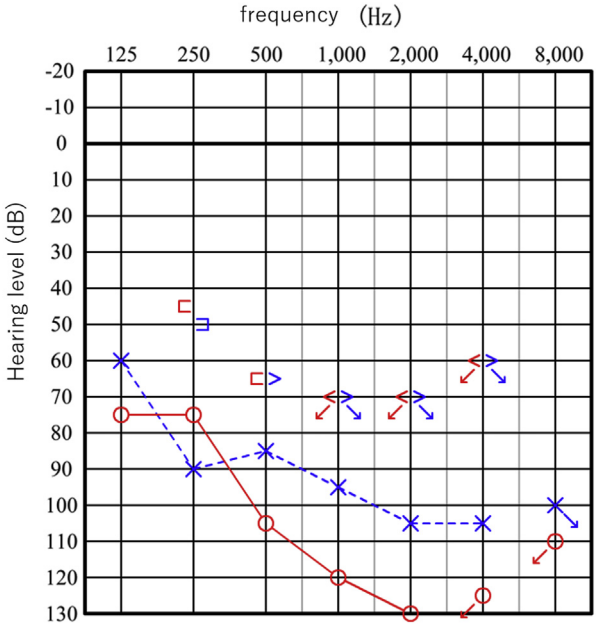

Fig. 2. Audiograms taken 10 months after the first visit, before CI.

examination that detected a heteroplasmic A8296G mitochondrial DNA mutation. Other mutations related to genetic hearing loss were not detected. Eighteen months after CI, the patient's speech perception measured using the Japanese version of CI2004 in silence and at a +10 dB signal-to-noise ratio (SNR10) had improved to 96% and 40% for words and 98% and 57% for sentences, respectively. Three years after CI, the patient's speech perception had further improved to 100% and 92% for sentences in silence and at an SNR10, respectively.

Discussion

We reported on a patient with an A8296G mitochondrial DNA mutation presenting bilateral moderate to severe hearing loss at high school age that progressed to bilateral severe hearing loss at age 22. The patient's low-tone hearing had been relatively well preserved compared to her hearing at high frequencies, but it eventually declined. The patient obtained good speech discrimination after CI surgery.

The patient's hearing loss began when she was a teenager. It affected primarily high frequencies and progressed to severe hearing loss at all frequencies by the age of 22. There has been no report on the precise course of hearing loss caused by the A8296G mitochondrial DNA mutation. Santorelli et al. [6] reported two families with a G8363A mitochondrial mutation that causes the same tRNA(Lys) deficiency. Some members of these families showed progressive SNHL, with the age of hearing loss onset varying from the teens to the fifties. Detailed information related to the clinical course of hearing loss has also been reported for other types of tRNA deficiency. The deficiency of tRNA(Leu) is primarily caused by an A3243G mitochondrial mutation. It has been reported that hearing loss occurs between 14 and 50 years of age. Most patients have bilateral progressive SNHL; the shape of the audiogram generally slopes at the beginning, gradually becoming flat [7]. Patients harboring tRNA(Ser) mutations of T7511C and A7445G have been reported to experience hearing loss in their second or third decades and show progressive hearing loss beginning at high frequencies [8,9]. From these findings, progressive hearing loss starting from high frequencies is considered a common feature in patients with a deficiency in their mitochondrial tRNA. The onset of hearing loss, however, varies according to the type of tRNA mutation. The age of onset of hearing loss in patients with tRNA(Leu) mutations is more variable than that of tRNA(Ser) mutations. Syndromic deafness associated with tRNA mutations such as A3243G is reported to be present in heteroplasmy with an atypical threshold effect [10], while non-syndromic deafness-associated tRNA mutations such as T7551C and A7445G are known to occur in homoplasy or in high levels of heteroplasmy [10]. These variations in mutation may result in the differences in the age of onset.

To the best of our knowledge, this is the first report of a patient with an A8296G mitochondrial mutation who underwent CI. The patient's postoperative speech perception score was very good. Most previous case reports also suggested that CI has favorable effects for patients with mitochondrial diseases [11]. It can be assumed that hearing loss associated with a mitochondrial disorder is more likely to be caused by cochlear dysfunction rather than retrocochlear abnormalities [12]. The animal model of chronic mitochondria dysfunction also suggested degeneration in the stria vascularis and the organ of Corti as a cause of hearing loss [13]. However, other reports analyzing the histopathology of the temporal bone with mitochondria tRNA mutations suggested additional retrocochlear impairments. A temporal bone study of A3243G mitochondrial mutations revealed severe degeneration of the spiral ganglion cells in addition to stria vascularis degeneration [14]. In cases with T7511C mutations, the primary cause of SNHL was considered due to severe degeneration of the spiral ganglion cells [8]. Moreover, pathological changes in the central nervous system including the central auditory pathway or psychomotor regression are reported in syndromic mitochondrial diseases such as MELAS [11,15]. Considering these findings, a longer follow-up is necessary to determine the outcome of CI.

Conclusions

We have detailed a case of a patient with severe sensorineural hearing loss due to an A8296G mitochondrial DNA mutation. She had bilaterally progressive hearing loss starting from high frequencies and her hearing at low frequencies declined thereafter. She underwent CI at the age of 22 and her postoperative speech discrimination was very good, indicating that patients with this mutation are good candidates for CI.

Conflicts of interest

None.

Ethical statement

This study was approved by the Institutional Review Board of the University of Tokyo Hospital (approval no. 2487). The requirement for informed consent was waived due to the retrospective nature of this study. The study was conducted according to the tenets of the Declaration of Helsinki and its revisions and Good Clinical Practice guidelines. No potential conflict of interest was reported by the author (s).

Financial disclosure

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Original article

Arima syndrome caused by *CEP290* specific variant and accompanied with pathological cilium; clinical comparison with Joubert syndrome and its related diseases

Masayuki Itoh [a,*], Shuhei Ide [b], Yuji Iwasaki [b], Takashi Saito [a], Keishi Narita [c], Hongmei Dai [a], Shinji Yamakura [d], Takeki Furue [e], Hirotsugu Kitayama [f], Keiko Maeda [g], Eihiko Takahashi [h], Kiyoshi Matsui [i], Yu-ichi Goto [a], Sen Takeda [c], Masataka Arima [b]

[a] *Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, Kodaira, Japan*
[b] *Division of Pediatrics, Tokyo Metropolitan Tobu Medical Center for Persons with Developmental and Multiple Disabilities, Tokyo, Japan*
[c] *Department of Anatomy and Cell Biology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Yamanashi, Japan*
[d] *Tsubasa Shizuoka, Shizuoka, Japan*
[e] *Department of Pediatric Nephrology, Hiroshima Prefectural Hospital, Hiroshima, Japan*
[f] *Department of Nephrology, Shizuoka Children's Hospital, Shizuoka, Japan*
[g] *Department of Pediatrics, Shizuoka Iryo-Fukushi Center, Shizuoka, Japan*
[h] *Division of Nephrology, Kanagawa Children's Medical Center, Yokohama, Japan*
[i] *Division of General Medicine, Kanagawa Children's Medical Center, Yokohama, Japan*

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Abstract

Objective: Arima syndrome (AS) is a rare disease and its clinical features mimic those of Joubert syndrome or Joubert syndrome-related diseases (JSRD). Recently, we clarified the AS diagnostic criteria and its severe phenotype. However, genetic evidence of AS remains unknown. We explored causative genes of AS and compared the clinical and genetic features of AS with the other JSRD.

Patients and methods: We performed genetic analyses of 4 AS patients of 3 families with combination of whole-exome sequencing and Sanger sequencing. Furthermore, we studied cell biology with the cultured fibroblasts of 3 AS patients.

Results: All patients had a specific homozygous variant (c.6012-12T>A, p.Arg2004Serfs*7) or compound heterozygous variants (c.1711+1G>A; c.6012-12T>A, p.Gly570Aspfs*19;Arg2004Serfs*7) in *centrosomal protein 290 kDa* (*CEP290*) gene. These unique variants lead to abnormal splicing and premature termination. Morphological analysis of cultured fibroblasts from AS patients revealed a marked decrease of the CEP290-positive cell number with significantly longer cilium and naked and protruded ciliary axoneme without ciliary membrane into the cytoplasm.

* Corresponding author at: Department of Mental Retardation and Birth Defect Research, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan.
E-mail address: itoh@ncnp.go.jp (M. Itoh).

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Conclusion: AS resulted in cilia dysfunction from centrosome disruption. The unique variant of *CEP290* could be strongly linked to AS pathology. Here, we provided AS specific genetic evidence, which steers the structure and functions of centrosome that is responsible for normal ciliogenesis. This is the first report that has demonstrated the molecular basis of Arima syndrome.

Keywords: Arima syndrome; Joubert syndrome; Joubert syndrome related diseases; *CEP290*; Cilium

1. Introduction

Arima syndrome (AS) (MIM #243910) has been mainly reported from Japan and its clinical features mimic those of Joubert syndrome (JS) (MIM #213300). Recently, we described diagnostic symptoms of AS; severe psychomotor delay from the early infantile period, cerebellar vermis agenesis (or hypoplasia), renal dysfunction with nephronophthisis, visual dysfunction due to retinitis pigmentosa and ptosis-like peculiar facial appearance [1,2]. Among them, the AS characteristic symptom is severely progressive renal failure from the infantile period. AS patients developed progressively renal failure and underwent dialysis or renal transplantation to survive in early childhood. AS may be a severe form of JSRD spectrum, seven AS patients previously demonstrated unique phenotypes [1]. Recently, among stacked up clinical reports of JS and its related diseases, it is established a disease entity of ciliopathy. Ciliopathy is defined as a disease group derived from cilia dysfunction and consist of clinically and genetically various diseases, including Joubert syndrome (JS) (MIM #213300) and JS-related diseases (JSRD) of Senior-Løken syndrome (SLS) (MIM #266900), COACH syndrome (CS) (MIM #216360), Leber congenital amaurosis (MIM #611755), Meckel-Grüber syndrome (MIM #24900), Bardet-Biedl syndrome (MIM #209900) and so on. The diseases commonly have the symptoms of severe psychomotor delay, cerebellar vermis agenesis, retinal dysfunction and dysplastic kidney [3,4]. On the other hand, 29 causative genes of ciliopathies have been reported [4–6]. The variants of these genes are closely related to dysfunction of the primary cilia.

Since the similarity of the phenotypes between AS and JS and other JSRD, we can speculate that AS is a member of JS and other JSRD. However, definitive genetic evidence of AS has never been submitted for making the diagnosis of ciliopathy. Here, we explored a causative gene of AS and considered its clinical spectrum and severity in JSRD from previous literatures.

2. Patients and methods

2.1. Patients

Previously, we experimented with 4 AS patients of 3 families from a Japanese nationwide survey. The AS diagnosis was done by two individual specialists with a revised criteria [1]. All patients were clinically diagnosed as AS, showing the characteristic 5 major features (Table 1, Supplementary Fig. 1).

All gene sequencing and fibroblast analyses, using provided the materials, were performed with permission by the ethical committee of the institutes and with informed consent of their parents.

2.2. Genetic examination

We obtained genomic DNAs from the blood cells of the 4 AS patients and the parents of their 3 families. We established fibroblast cell culture from 3 patients of AS1 II-1, AS1 II-2 and AS3 II-1 in Fig. 1. Using cultured cells, we performed genetic analysis, cytopathology and expression studies.

For genetic variation study, we conducted whole-exome sequencing in the genomic DNAs on a HiSeq2000 sequencer (Illumina, San Diego, CA) with 101 bp paired-end reads and 6 bp index reads. The sequence data were mapped to the human reference genome (hg19) with BWA (http://bio-bwa.sourceforge.net/index.shtml). Single base substitutions and indels was detected by SAMTOOLS (http://samtools.sourceforge.net/) and annotated to dbSNP. The mean depth of coverage was 100 reads, with approximately 95% of all coding exons being covered by 10× or more reads. Out of all variants within exons and 20-bp intronic regions from the exon–intron boundaries, those registered in dbSNP135 (http://www.ncbi.nlm.nih.gov/projects/SNP/) and 1000 Genomes (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) were removed. According to autosomal recessive inheritance pattern of AS, homozygous or compound heterozygous variants were then picked up. To confirm those variants, we performed direct Sanger sequencing of genomic DNA derived from all patients and their parents, using ABI PRISM 310 genetic analyzer. In addition, we performed subcloning an reverse transcriptase-PCR (RT-PCR) product amplified from total RNA of fibroblasts into a pDNA-TOPO vector (Invitrogen, Carlsbad, CA) and synthesized each cDNA by SuperScript II reverse transcriptase (Life Technology, Waltham, MA) with a random primer. Then, we checked patients' cDNA sequences with Sanger sequencing, to confirm each

Table 1
Clinical features of our patients.

	Patient	AS1 II-1	AS1 II-2	AS2 II-1	AS3 II-1
	Age (y)	25	21	14	28
	Sex	F	F	F	F
Major symptoms*	Severe psychomotor delay	+	+	+	+
	Cerebellar vermis agenesis or hypoplasia	+	+	+ + Encephalocele	+
	Progressive renal dysfunction at infantile or adolescent period	+ Dialysis/18y	+ Dialysis/17y	+ Dialysis/11y	+ Dialysis/7y, TP/14y
	Visual dysfunction from the early stage	+ RP	+ RP	+ RP	+ RP
	Unilateral or bilateral ptosis-like facial appearance	+	+	+	+
Associated symptoms*	Peculiar face: hyperterolism, saddle nose, large mouth	+	+	nd	+
	Other symptoms: dehydration, growth retardation, fever of unknown origin	+	+	nd	+
Supportive laboratory examinations*	Blood examination: anemia, high level of BUN and/or creatinin	+	+	+	+
	Urinary examination: hypo-osmolality, high-level of β2-microgloblin and/or NAG	+	+	+	+
	Electroretinography: no response or low voltage	nd	+	+	+
	Kidney CT, MRI or echo scan: polycystic kidney	+	+	+	+
	Kidney biopsy: nephronophthisis	nd	nd	+	+
	Abdominal echo scan: fatty liver, liver enlargement, liver cirrhosis, etc	nd	nd	nd	nd
Other symptoms		Abnormal EEG	Epilepsy/16y	Epilepsy/3y	Epilepsy/3y

Abbreviations: y, years; F, female; RP, retinitis pigmentosa; TP, renal transplantation; nd, not done or not described; EEG, electroencephalography.
* Clinical criteria and symptoms due to a reference [1].

variant in the different alleles. The primer sequences and condition were shown in Supplementary Method.

2.3. Cell biological analysis

For cell biology studies, we used the cultured fibroblasts of our 3 patients (AS1 II-1, AS1 II-2 and AS3 II-1) and two healthy controls. We performed immunoblotting by the prepared protein extracts with the antibodies of CEP290 (1:100, Abcam) and β-actin (1:5000, Sigma-Aldrich, St. Louis, MO). We confirmed a specific band with a luminescent image analyzer (ImageQuant LAS 4000 mini; GE Healthcare) and an equal β-actin expression level of each lane for normalization.

In order to characterize morphological profiles of primary cilia in AS patients, we performed immunocytochemistry on the primary cultured fibroblasts with the primary antibodies against CEP290 (1:100, Abcam, Cambridge, UK) and acetylated alpha-tubulin (1:100, Sigma-Aldrich). After staining, we randomly collected 500 cells and measured the length of the acetylated tubulin-immunopositive rod-like structure as cilia in each cell, using ImageJ software, version 1.45 (NIH, Bethesda, MD). Then, we calculated the mean length and standard deviation in each cell, and analyzed with variance and statistically significance of $P < .05$ with Student *t*-test.

For transmission electron microscopy analysis, fibroblasts were fixed and processed as previously described [7]. After the preparation, imaging was performed with a H-7500 electron microscope (Hitachi High-Tech, Tokyo, Japan).

3. Results

3.1. Clinical characteristics

Clinical profiles of 4 AS patients were exhibited in Table 1. All had normal delivery, but showed hypotonia, ptosis, developmental delay, retinitis pigmentosa, brain malformation with molar tooth sign on MRI and renal dysfunction in the infantile period. They revealed no response of electroretinogram and abnormal electroencephalogram with or without epilepsy. Their renal dysfunction developed renal failure until the late childhood or adolescence and requested dialysis or transplantation. In our cases, AS2 II-1 showed the most severe phenotypes, such as malformed brain of occipital encephalocele and early progressive renal failure.

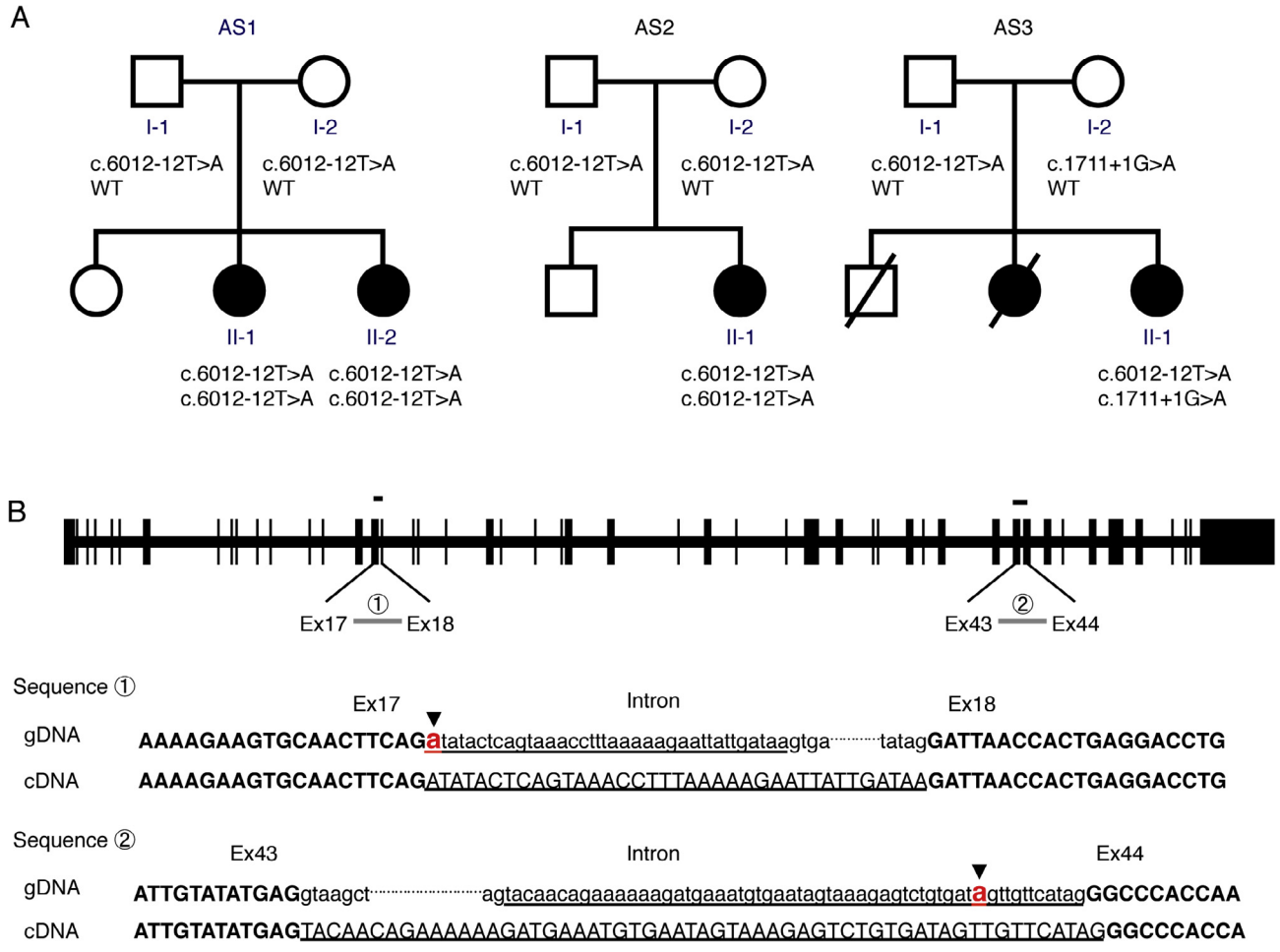

Fig. 1. Mutation analyses of patients with Arima syndrome. (A) Family pedigrees reveal autosomal recessive inherited pattern. All patients diagnosed with Arima syndrome have a commonly homozygous mutation or compound heterozygous mutation. (B) The loci and sequences of their variants in *CEP 290* gene are indicated in H. A sequence ① of exons 16 and 17 and intron 16 reveals a variant of c.1711+1G>A (arrowhead and bold character) in genomic DNA. As a result, a partial intron sequence inserts in between exon 16 and 17. A sequence ② of exons 43 and 44 and intron 43 reveals a mutation of c.6012-12T>A (arrowhead and bold character) in genomic DNA. As a result, a partial intron sequence inserts in between exon 43 and 44. The underlines indicate retained intronic sequences of genomic DNA. Accession Numbers are NG_008417 for genome DNA sequencing and NM_025114.3 for complementary DNA sequencing. gDNA, genomic DNA; cDNA, complementary DNA.

3.2. Specific variants of CEP290

From data of whole exome analysis, we could narrow down the candidates to 68 genes. Moreover, the common candidate variants of all patients and parents were found in only *centrosomal protein 290 kDa* (*CEP290*) gene. By Sanger sequencing, we confirmed that all patients had a homozygous mutation (c.6012-12T>A, p.Arg2004Serfs*7) in AS1 II-1, AS1 II-2 and AS2 II-1 and a specific compound heterozygous mutation (c.1711+1G>A; c.6012-12T>A, p.Gly570Aspfs*19;Arg2004Serfs*7) in AS3 II-1 in *CEP290* gene (Fig. 2, Supplementary Figs. 2–4). In addition, their parents had the same heterozygous mutations of the patients (Supplementary Fig. 2). The variants have not been reported in dbSNP135, 1000 Genomes, LOVD3.0 (http://www.lovd.nl/3.0/home) and Japanese SNPs database (JSNP Database (http://snp.ims.u-tokyo.ac.jp/)). In addition, we checked that the variants were not in the following database; CEP290base (medgen.ugent.be/cep290base), ExAC (http://exac.broadinstitute.org/), EVS (http://evs.gs.washington.edu/EVS/) and HGVD (http://www.hgvd.genome.med.kyoto-u.ac.jp/). From the cDNA sequencing study, the variants we discovered were found in the introns and resulted in abnormal splicing and premature termination (Supplementary Figs. 1–3). The NCBI accession numbers for the *CEP290* sequence reported in this paper were NG_008417.1 for human genomic DNA and NM_025114.3 for human cDNA.

3.3. Comparison of clinical features of Arima syndrome, Joubert syndrome, Senior-Løken syndrome, COACH syndrome, and Dekaban syndrome

We reviewed clinical features and genetic variants of AS, JS, SLS, CS and Dekaban syndrome (DS) as com-

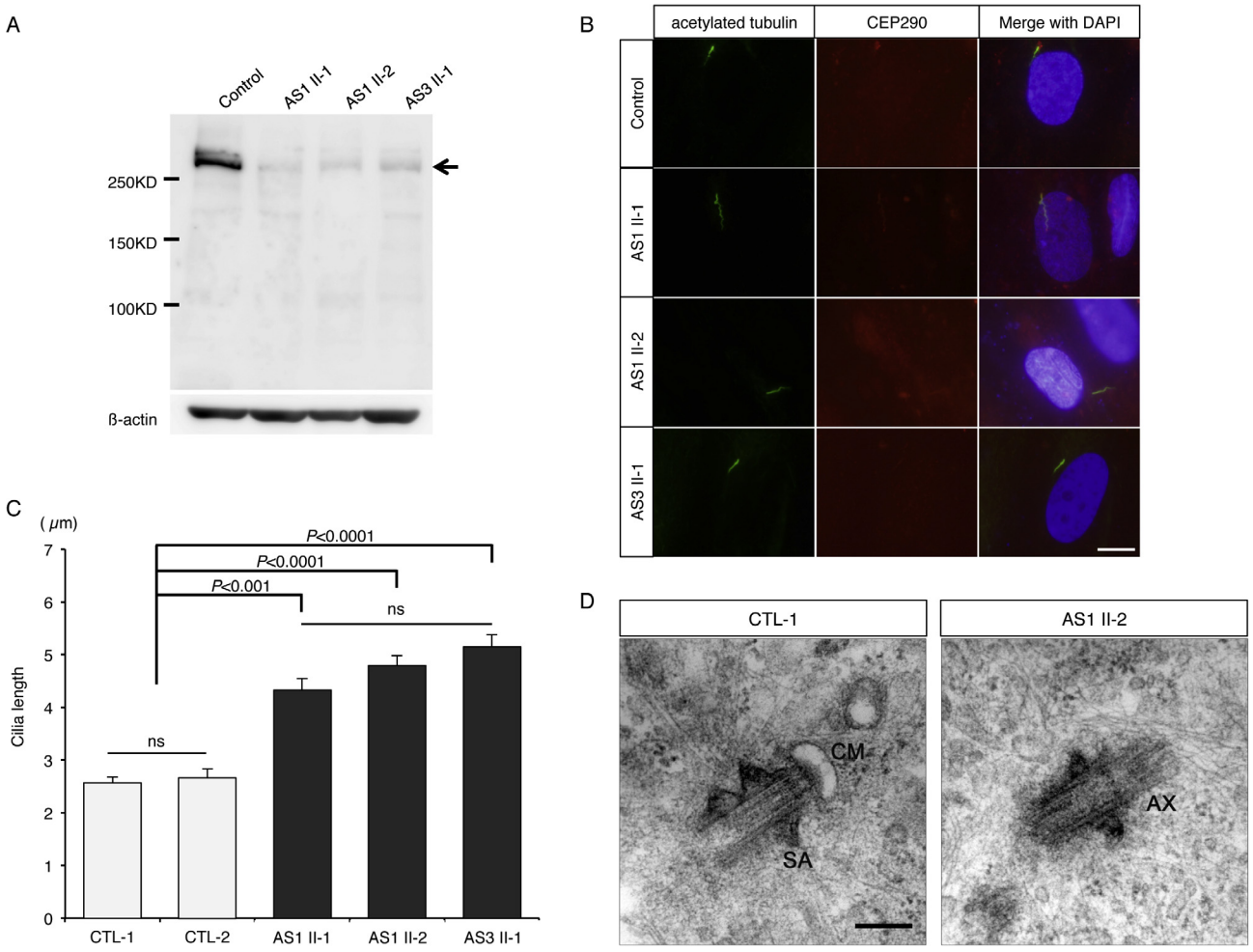

Fig. 2. Cytopathology of Arima syndrome. (A) CEP290 protein of patient fibroblasts has a relatively lighter molecular weight than controls and reveal degradation. (B) Immunocytochemistry of Arima syndrome patient fibroblasts demonstrates an elongated cilium, compared with controls. (C) All cilia of patient fibroblasts are significantly longer than those of controls, although there are no differences between those of patient fibroblasts. (D) Transmission electron microscopy of basal bodies in human skin fibroblasts. In control fibroblasts, ciliary membrane was docked at the distal end of the basal body at an early stage of ciliogenesis. By contrast, Arima syndrome fibroblasts exhibited failure in the membrane docking. ns, not significant; CTL, control; SA, subdistal appendage; CM, ciliary membrane; AX, axoneme. Scale bar in B, 10 μm. Scale bar in D, 250 nm.

mon phenotypes diseases, having molar tooth sign (MTS), abnormal eye and respiratory movement and malformed kidney, from previous reports (Table 2). Hypotonia was a major symptom of AS and JS. AS phenotypes were relatively severe than those of JS. SLS might be high frequency of anemia. CS showed characteristically coloboma and liver fibrosis, while the features often were noticed in other syndromes. It was well known that DS was essentially retinopathy. However, clinical description was very few. Genetically, the causative genes shared cilia-related genes, especially *CEP290*, *NPHP1*, *RPGRIP1L*, *NEK8* and *TMEM67*. The essential features of AS were cerebellar vermis agenesis (showing MTS on imagings), severe psychomotor retardation from the infantile period, visual impairment with retinal degeneration and progressive renal failure (pathologically nephronophthisis) from childhood as a previously reported criteria. Therefore, AS may be a member of JSRD, as most phenotypes of AS overlap with JS and other JSRD. Moreover, we can permit to mention that AS is the most severe form of JSRD about renal function and psychomotor development from the early life-stage.

3.4. Cell morphological analysis

In AS patient fibroblasts, CEP290 showed very weak immunopositivity and incomplete degradation (Fig. 2A). Immunoblotting demonstrated a faint band in every lane from AS patient against a specific band in control. On the other hand, the immunocytochemistry identified ciliary projections in both control and AS patient fibroblasts (Fig. 2B). By measuring the length of the acetylated-tubulin immunopositive projections, the length of primary cilia in AS fibroblasts assumed to be significantly longer than that of control

Table 2
Clinical features of patients.

		Arima syndrome	Joubert syndrome	Senior-Løken syndrome	COACH syndrome	Dekaban syndrome
Psychomoter development		Severe delay from the early infantile period	Severe delay to normal	Various	Various	Severe delay from the early infantile period
Brain	Molar tooth sign	+	+	+	+	+
	Cerebellar vermis agenesis	+	+	+	+	+
	Brainstem malformation (hypoplasia)	+	+	+	–	+
	Hypotonia	+	+	Not described	Not described	Not described
Eye/ Retina	Retinopathy (Retinitis pigmentosa)	+	+	+	–	+
	Coloboma	–	+/–	–	+	–
	Abnormal eye movement (Nystagmus, Strabismus, dysrexia)	+	+	+	+	Not described
Kidney	Nephronophthisis	+	+	+	+	+
	Polycystic kidney	+	+	–	–	+
Abnormal respiratory		+	+	+	+	Not described
Others		Ptosis-like appearance (daily alteration)	Various	Anemia	Liver fibrosis	Not described
Genetics		*CEP290*: c.6012-12T>A (p. Arg2004Serfs*7); c.1711+1G>A (p.Gly570Aspfs*19)	*AHI1*, *NPHP1*, *CEP290*, *RPGRIP1L*, *NEK8*, *TMEM67*, *TTC21B*, *TMEM231*	*IQCB1*, *NPHP1*, *INVS*, *NPHP3*, *NPHP4*, *CEP290*, *SDCAAG8*	*TMEM67*, *NEK8*, *CC2D2A*, *RPGRIP1L*	Not reported
Prognosis		Usually early death for renal failure	Various, but not early death (depend on renal or respiratory condition)	Various, but not early death (depend on renal or respiratory condition)	Various, but not early death (depend on renal or respiratory condition)	Not described
References		[1–5]	[3,6,–10]	[3,4,6,10,11]	[3–7,9,10,12,13]	[3–5,14]

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fibroblasts (Fig. 2B and C). These findings were consistent among the three individual AS cells. In these cells, CEP290 expression was drastically decreased and its level varied among AS patients.

In addition, we ultrastructurally identified a peculiar image of a basal body structure (Fig. 2D). In control fibroblasts, the distal tip of the basal body was identified by the subdistal appendage (SA), which attached to the nascent ciliary membrane (CM; CTL-1 in Fig. 2D). On the contrary, some axonemes in AS patient fibroblast (AS 1 II-2 in Fig. 2D) directly protruded into the cytoplasm without any accompanying membranous structures.

4. Discussion

Since the first report of AS in 1971, over 10 literatures for AS has been mainly reported from Japan. It has been considered that AS is a part of JS or similarly to DS [8]. Clinically, DS might specify severe retinopathy [9]. AS is characterized by progressive renal failure and severe mental dysfunction from early childhood. Especially, renal dysfunction of AS patient, whose pathology shows nephronophthisis, is the most severe and develops dialysis as early as 20 years of age [2,10]. AS can be thought to be the severe form of JSRD (Table 2).

AS causative gene had been never found, while many variants of cilia-related genes were discovered in the other JSRD. Here, we revealed specific *CEP290* variant as AS causative gene, which incompletely degraded its product. All AS patients shared a c.6012-12T>A in *CEP290*. In the huge SNPs databases of dbSNP135, 1000 Genomes, LOVD3.0 and JSNP Database, both variants of c.6012-12T>A and c.1711+1G>A are very rare. From the ExAC database, c.6012-12T>A (p.Arg2004Serfs*7) is found 3/8614 allele (Allele frequency 0.0003483) only in East Asian population. A c.6012-12T>A (p.Arg2004Serfs*7) may also be a very rare variant in Japanese, although another study revealed that the allele frequency was 1/270 allele (Allele frequency 0.0037) in a previous report [11]. The reported patients with the same mutation as JS or MTS had never described clinical course and biological data [6,11]. Therefore, we could not discuss about phenotype comparison of their patients and our patients. The specific variant may be the reason that AS has been mainly reported from Japan. The rare homozygous mutation can be speculated to be pathogenesis of AS. Moreover, it is speculated that the heterozygous mutation does not form any phenotypes as those of their parents. AS patients commonly had the homozygous or heterozygous variant of c.6012-12T>A. This variant may be necessary in the formation of AS pathology. In addition, a compound heterozygous mutations containing c.1711+1G>A (p.Gly570Aspfs*19) was found in only one case of Leber congenital amaurosis [12]. This fact can support that a variant of c.6012-12T>A (p.Arg2004Serfs*7) is important to form AS phenotype. However, as the specific variants of CEP290 linked to AS, it may be possible to form another severe phenotype of JSRD by unique variants of ciliopathy genes other than CEP290.

The gene of *CEP290* is located on 12q21.32 and encodes a protein with various functional domains [13,14]. CEP290 constructed a distal appendage of the basal body of cilia and acetylated-tubulin confirmed maturation of ciliary axonemes. Considering the mutation of CEP290 involves the 12th CC domain and MYO-tail homology domain, which possibly make contact with the unconventional myosin, abnormal ciliogenesis can be explained by the impaired recruitment of membranous components [11,15]. A unique variant of AS resulted in insertion of partial intron 43 and premature termination. The C-terminus of CEP290 can bind itself through homotypic interaction, and the C- and N-termini are capable of binding each other for dimerization and maintenance of the basal body structure [16]. Therefore, the C-terminus structure may be important in forming AS pathology. In addition, disruption of the MYO-tail homology domain may lead to microtubule-associated transport failure. Taken together, its molecular pathology reflected the AS fibroblast phenomenon. On the other hand, the mRNA produced by the variants of c.1709C>G, p.Ser570Ter in LCA patient and c.1711+5G>A in JS patient might be targeted for nonsense-mediated decay [13,17]. The variant of c.1711+1G>A could resemble null mutation, because of the near location in the patients. Thus, it might be linked to AS pathology.

Many *CEP290* mutations have been reported in various ciliopathies of JS, SLS, Leber congenital amaurosis, Meckel-Grüber syndrome, Bardet-Biedl syndrome [18,19]. Clinical features of these disorders overlap and affect multiple organ systems, most commonly the brain, retina and kidney. *CEP290* mutations cause a broad spectrum of phenotypes of JS and JSRD.

It is an important process for normal ciliogenesis and axonemes extending to bind cell membrane with ciliary basal body assembled with CEP290 and recruiting Rab8a, a small GTPase [20,21]. The complex with CEP290 and Rab8a is needed for ciliary membrane elongation at centrosomes and cilia [20–22]. However, AS patient fibroblasts revealed an extension of axonemes without the enwrapped ciliary membrane. The phenomenon indicates that initial docking of basal body to the cell membrane may be disrupted in AS fibroblasts, and it can be related to the abnormally long cilium, whose regulation of length is controlled by CEP290 complex. Interestingly, the *Cep290* disruptions develop progressive degeneration of cilia and elongation of cilia took place [23]. Recently, renal epithelial cells of JS patients with *CEP290* pathogenic mutations, whose products were decomposed, showed cilium elongation

[24]. CEP290 degradation may be link to the phenomenon of cilium elongation.

On the other hand, primary cilium has been known as sensory organella, which is the origin of intracellularly physiological and chemical signals, such as developmental sonic hedgehog signaling, cell migration and cell cycling [13]. Centrosomes directly act on cell division, assemble microtubules, transport materials in cells and maintain cell structure [25,26]. It has been reported that neuropeptide FF receptor 2 of the primary cilia controls the flow of cerebrospinal fluid [27]. These structural and functional disruptions lead to forming common symptoms of ciliopathies.

Here, we revealed the molecular basis of Arima syndrome. Our next goal is to elucidate the molecular mechanism of abnormal ciliogenesis in AS patients from the perspective of CEP290 function.

Acknowledgments

Two variations of *CEP290*, which we specifically discovered, are registered in Leiden Open Variation database (LOVD) v.3.0 (http://www.lovd.nl/3.0/home). The variant IDs are 0000066685 of g.88462434T>A (c.6012-12T>A) and 0000066686 of g.88512259G>A (c.1711+1G>A).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.braindev.2017.11.002.

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