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The Laryngoscope

Subgroups of Enlarged Vestibular Aqueduct in Relation to *SLC26A4* Mutations and Hearing Loss

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Objectives/Hypothesis: To investigate possible association of hearing loss and *SLC26A4* mutations with the subgroups of enlarged vestibular aqueduct (EVA) morphology in Japanese subjects with hearing loss.

Study Design: Retrospective multicenter study.

Methods: Forty-seven subjects who had vestibular aqueduct with midpoint diameter >1 mm by computed tomography of the temporal bone were enrolled at multiple sites across Japan, and DNA samples and clinical data were collected. EVA morphology was classified into four subgroups by the pattern of enlargement: aperture, aperture and midpoint, midpoint, and borderline enlargement. Venous blood DNA samples were subjected to polymerase chain reaction–based direct sequencing of all exons and exon–intron boundaries of the *SLC26A4*.

Results: Four novel *SLC26A4* mutations were identified in the present study. *SLC26A4* mutations were detected in almost all subjects with aperture, aperture and midpoint, and midpoint enlargement. In contrast, 71% of subjects with borderline enlargement had no *SLC26A4* mutation. No significant difference was found in the distribution of truncating and nontruncating *SLC26A4* mutations between the EVA subgroups. In addition, no significant correlation was observed between the EVA subgroups and hearing levels, incidence of hearing fluctuation, or progression of hearing loss.

Conclusions: Subgroups of EVA morphology were significantly correlated with the presence or absence of *SLC26A4* mutation. In a subgroup analysis of subjects with *SLC26A4* mutations, however, differences in the EVA subgroups were not correlated with *SLC26A4* genotypes or characteristics of hearing loss.

Key Words: Enlarged vestibular aqueduct, Pendred syndrome, DFNB4, *SLC26A4*, computed tomography, hearing loss.

Level of Evidence: NA

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INTRODUCTION

Enlarged vestibular aqueduct (EVA) is one of the most common inner ear deformities, often identified by computed tomography (CT) in subjects with hearing loss.[1–5] The shape and size of the EVA differ between subjects. As such, a variety of radiographic criteria to define EVA have been published. Valvassori and Clemis[6] defined EVA as a vestibular aqueduct ≥1.5 mm at the midpoint diameter. Jackler and De La Cruz[7] developed a criterion of a midpoint diameter >2.0 mm, whereas Levenson and colleagues[8] proposed a cutoff of 2.0 mm at the external aperture diameter. Okumura et al.[9] suggested an external aperture diameter >4.0 mm. Madden et al.[1] considered external aperture diameter >2.0 mm and midpoint diameter >1.5 mm as definitive, and midpoint diameter of 1.0 to 1.5 mm as borderline enlargement. Vijayasekaran et al.[10] advocated the criteria of 0.9 mm midpoint diameter or 1.9 mm external aperture diameter.

Mutations in the *SLC26A4* have been identified as a major cause of vestibular aqueduct anomalies. *SLC26A4* mutations are known to cause Pendred syndrome (Mendelian Inheritance in Man [MIM] #274600) and nonsyndromic sensorineural deafness autosomal recessive type 4 (DFNB4, MIM #600791).[11–14] Some researchers have identified a correlation between *SLC26A4* mutations, EVA, and hearing loss, whereas others report no significant relationship among *SLC26A4* genotype and these phenotypes.[15] Previous

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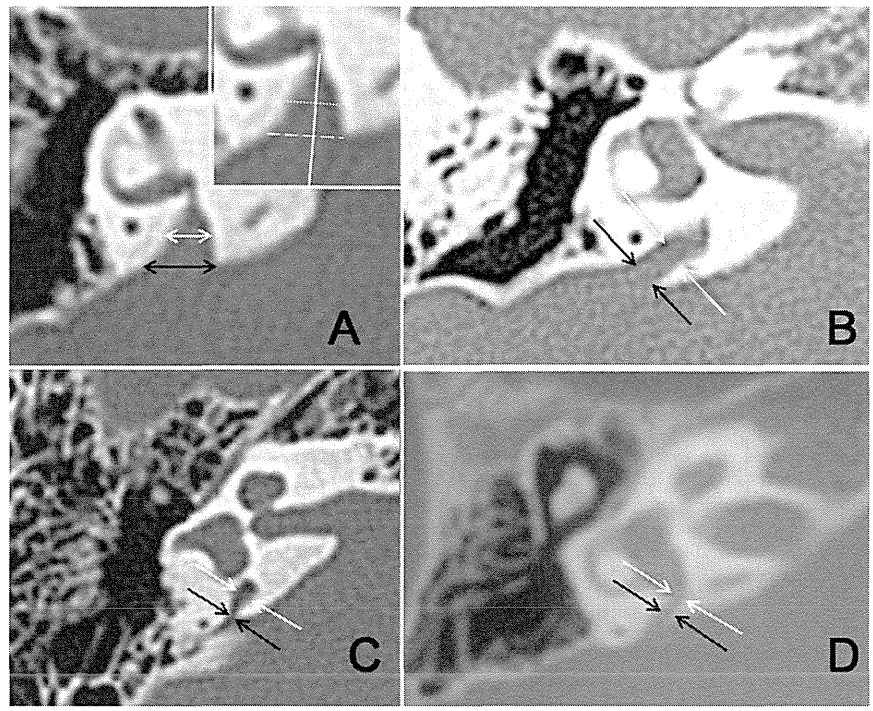

Fig. 1. Typical temporal bone computed tomographic images of the enlarged vestibular aqueduct subgroups. (A) Aperture enlargement. (B) Aperture and midpoint enlargement. (C) Midpoint enlargement. (D) Borderline enlargement. The midpoint and external aperture of the vestibular aqueduct are indicated by white and black arrows, respectively. As shown in the inset of A, the midpoint diameter (dotted line) and aperture diameter (dashed line) were measured perpendicular to the long axis (solid line) of the vestibular aqueduct.

studies have not evaluated the relationship between *SLC26A4* mutations and clinical features of hearing loss taking into consideration morphologic variations of the EVA. We conducted a multicenter study and differentiated subjects into subgroups according to vestibular aqueduct midpoint and external aperture diameters to examine a possible relationship between subgroups of EVA morphology, *SLC26A4* mutations, and hearing loss.

MATERIALS AND METHODS

We enrolled 47 bilateral EVA subjects with unilateral or bilateral sensorineural hearing loss of unknown causes (mean age = 13.5 years, range = 0–56 years; 33 children and 14 adults; 17 males and 30 females), and collected DNA samples and clinical data. Specifically, subjects whose bilateral vestibular aqueduct midpoint diameter was ≥1 mm on temporal bone CT scans were included. The midpoint and external aperture diameters were measured perpendicular to the long axis of the vestibular aqueduct on the transverse plane, as shown in the upper right-hand inset in Figure 1A. Subjects were classified into the following four subgroups based on the morphologic characteristics of the vestibular aqueduct according to the criteria in Table I: aperture enlargement, aperture and midpoint enlargement, midpoint enlargement, and borderline enlargement.

For mutation analysis, genomic DNA was extracted from venous blood and subjected to polymerase chain reaction–based direct sequencing of the exons and exon–intron boundaries of the *SLC26A4* (GenBank NG_008489). For the purpose of this study, frameshift, splice site, and nonsense mutations were categorized as "truncating," and missense mutations as "nontruncating" mutations. Novel variants were defined as pathogenic if they 1) were nonsynonymous; 2) demonstrated low carrier rates (<1%) in 96 normal control Japanese subjects, absence in database Exome Variant Server[16] and dbSNP,[17] and high amino acid conservation among various mammalian species; and 3) were detected as heterozygous in association with the other allele with another heterozygous mutation already reported as pathogenic. Alteration of splice site was predicted by NNSPLICE.[18] Subjects with *SLC26A4* mutations were analyzed for degree of hearing loss, fluctuations in hearing acuity, and progression of hearing loss to assess the relationship between these hearing parameters and EVA subgroups. Subjects underwent conditioned orientation reflex or conventional pure-tone audiometry, depending on their ages. Auditory steady-state response measurements were utilized for five subjects who did not receive any of these audiometric tests.

TABLE I.
Criteria for the Subgroups of Enlarged Vestibular Aqueduct.

Enlarged Vestibular Aqueduct Subgroup	Midpoint Diameter	External Aperture Diameter
Aperture enlargement	≥1.5 mm	Wider than midpoint
Aperture and midpoint enlargement	≥1.5 mm	Equal to midpoint
Midpoint enlargement	≥1.5 mm	Narrower than midpoint
Borderline enlargement	1.0 mm to <1.5mm	1.0 mm to <1.5 mm

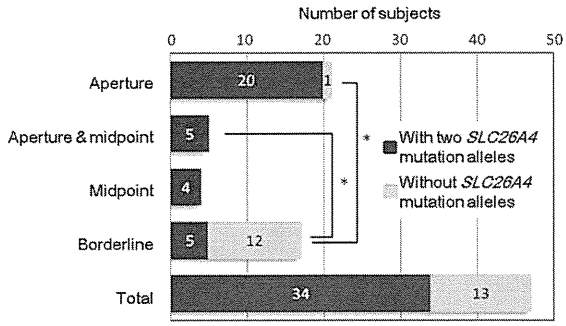

Fig. 2. Number of subjects with or without *SLC26A4* mutation alleles in each enlarged vestibular aqueduct subgroup. *Significant difference ($P < .0125$).

Hearing level was evaluated based on averages at 500, 1,000, 2,000, and 4,000 Hz (slight, 26–40 dB; moderate, 41–60 dB; severe, 61–80 dB; profound, ≥81 dB) according to the World Health Organization Grades of Hearing Impairment.[19] Subjects were considered to have fluctuating hearing loss if they had at least one bout of aggravation of hearing loss and recovery (at least 15 dB in one frequency). Subjects were considered to have progressive hearing loss if they showed aggravation of hearing loss by 10 dB or more at one or more frequencies within a 10-year interval. Statistical significance was assessed using the Fisher exact test.

All procedures were approved by the Ethics Review Committee of National Hospital Organization Tokyo Medical Center, Japan and other participating institutions, and were conducted only after written informed consent had been obtained from each subject or from the parents of the subjects.

RESULTS

Subgrouping of EVA and Its Association With SLC26A4 *Mutations*

Figure 1 shows typical CT findings in subjects with aperture enlargement (Fig. 1A), aperture and midpoint enlargement (Fig. 1B), midpoint enlargement (Fig. 1C), and borderline enlargement (Fig. 1D). Among 47 subjects, 21 (44%) were classified with aperture enlargement, 17 (36%) with borderline enlargement, five (11%) with aperture and midpoint enlargement, and four (9%) with midpoint enlargement (Fig. 2). All subjects had the same subgroup of enlargement bilaterally.

Genetic analysis of the 47 subjects showed that 34 (72%) had two *SLC26A4* mutation alleles (Table II), and the other 13 (28%) had no *SLC26A4* mutation alleles. None had a single *SLC26A4* mutation allele. The 34 subjects with two *SLC26A4* mutation alleles were diagnosed with Pendred syndrome or DFNB4. The majority of these subjects had aperture enlargement (n = 20, 59%), followed by aperture and midpoint enlargement (n = 5, 14%), borderline enlargement (n = 5, 14%), and midpoint enlargement (n = 4, 12%; Fig. 2). Conversely, most of the subjects without *SLC26A4* mutation alleles had borderline enlargement (n = 12, 91%), whereas the one remaining subject (8%) had aperture enlargement. The frequency of subjects without *SLC26A4* mutation alleles in the borderline enlargement subgroup was significantly higher than in the aperture enlargement and aperture and midpoint enlargement subgroups ($P < .0125$). It tended to be higher than in the midpoint enlargement subgroup, but this difference was not statistically significant ($P = .021$), probably due to the small number of subjects in the midpoint enlargement subgroup (n = 4).

SLC26A4 *Mutations and Genotypes in Association With EVA Morphology in Subjects With Pendred Syndrome or DFNB4*

The types and locations of all the *SLC26A4* mutations in 34 subjects with Pendred syndrome or DFNB4 are shown in Table II and Figure 3. Five splice site mutations (c.601-1G>A [intron 5], c.919-2A>G [intron 7], c.1614+1G>A [intron 14], c.1708-32_1708-16del [intron 15], c.1707+5G>A [intron 15]), one nonsense mutation (p.L743X), two insertion/deletion mutations (p.S551Ffs13, p.Q705Wfs18), and 14 missense mutations (p.S28G, p.P76S, p.A372V, p.N392Y, p.R409H, p.T410M, p.T527P, p.I529S, p.Y556C, p.V659L, p.D669E, p.F692L, p.T721M, p.H723R) were detected. These included four novel mutations, p.S28G (c.82A>G), p.D669E (c.2007C>A), p.F692L (c.2074T>C), and c.1708-32_1708-16del (marked with ** in Table II), based on the criteria for novel mutations in the present study (described in Materials and Methods). Electropherograms of the novel mutations and conservation of the amino acid residues among various species are shown in Figure 3B and C. NNSPLICE predicted c.1708-32_1708-16del to decrease the probability of an acceptor site at exon 16 from 0.49 (for a normal allele) to 0.19 (for a mutation allele), which is likely to cause aberrant splicing (Fig. 3C).

The list of subjects with two *SLC26A4* mutation alleles is shown in Table II. Analysis of genotypes of *SLC26A4* mutation alleles in these subjects showed that 20 (59%) had nontruncating/nontruncating genotypes, 13 (38%) had nontruncating/truncating genotypes, and 1 (3%) had truncating/truncating genotypes (Fig. 4A). Comparison of the incidence of each genotype found no significant statistical difference between the subgroups of EVA morphology ($P = 1.000$).

Characteristics of Hearing Loss in Association With EVA Morphology in Subjects With Pendred Syndrome or DFNB4

The hearing levels, incidence of hearing fluctuation, and progression of hearing loss in subjects with two *SLC26A4* mutation alleles are shown in Table II. The relation between the hearing level and EVA morphology was examined in the ears of 34 subjects (68 ears; Fig. 4B). Thirty-four ears (50%) had profound hearing loss in total. No significant differences in the hearing levels were detected between the subgroups of EVA morphology ($P = .462$). To exclude the effect of aging in this analysis, we also stratified the subjects into two groups (age 0–9 and ≥10 years) and conducted the same analysis. These analyses also demonstrated the same results, indicating that the difference in ages among subgroups did

TABLE II.
Types of *SLC26A4* Mutations and Characteristics of Hearing Loss in 34 Subjects With Pendred Syndrome or DFNB4 by EVA Subgroups.

			Allele 1			Allele 2						
EVA Morphology	Age at Deafness Diagnosis, yr	Age, yr	Exon/Intron	DNA Change	Amino Acid Change or Splicing Mutation	Exon/Intron	DNA Change	Amino Acid Change or Splicing Mutation	T/N	Hearing Level, R/L, dBHL*	Fluctuation of Hearing	Progression of Hearing Loss
Aperture enlargement	0	1	Intron 15	c.1707+5G>A	Splice site mutation	19	c.2106–2110dup5	p.Q705Wfs18	T/T	90/70†	–	+
	0	33	15	c.1652insT	p.S551Ffs13	19	c.2168A>G	p.H723R	T/N	95/95	–	+
	2	6	Intron 7	c.919-2A>G	Splice site mutation	19	c.2168A>G	p.H723R	T/N	53.75/63.75	–	+
	0	27	Intron 7	c.919-2A>G	Splice site mutation	19	c.2168A>G	p.H723R	T/N	98.75/100	+	+
	0	1	Intron 15	c.1707+5G>A	Splice site mutation	19	c.2168A>G	p.H723R	T/N	85‡	Unknown	+
	0	31	Intron 5	c.601-1G>A	Splice site mutation	19	c.2168A>G	p.H723R	T/N	73.75/60	+	+
	3	11	Intron 5	c.601-1G>A	Splice site mutation	19	c.2168A>G	p.H723R	T/N	70/87.5	+	+
	0	4	Intron 15	c.1707+5G>A	Splice site mutation	2	c.82A>G**	p.S28G**	T/N	61.25/61.25	–	Unknown
	0	35	Intron 5	c.601-1G>A	Splice site mutation	10	c.1229C>T	p.T410M	T/N	80/73.75	+	+
	3	12	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	82.5/106.25	+	+
	3	3	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	62.5/73.75	–	–
	0	4	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	55/70	+	–
	0	2	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	37.5‡	Unknown	Unknown
	0	1	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	102.5/115§	–	–
	0	0.5	10	c.1229C>T	p.T410M	19	c.2228T>A	p.L743X	N/N	73.75‡	Unknown	Unknown
	0	1	9	c.1115C>T	p.A372V	10	c.1226G>A	p.R409H	N/N	92.5‡	–	–
	0	20	19	c.2168A>G	p.H723R	14	c.1579A>C	p.T527P	N/N	97.5/101.25	–	–
	0	4	15	c.1667A>G	p.Y556C	14	c.1579A>C	p.T527P	N/N	77.5/75	–	+
	0	6	3	c.266C>T	p.P76S	14	c.1579A>C	p.T527P	N/N	17.5/93.75	–	+
	0	9	10	c.1174A>T	p.N392Y	19	c.2162C>T	p.T721M	N/N	103.75/110	+	+
Aperture and midpoint enlargement	0	15	Intron 15	c.1708-32_1708-16del**	Splice site mutation**	19	c.2168A>G	p.H723R	T/N	76.25/91.25	+	+
	0	9	Intron 7	c.919-2A>G	Splice site mutation	17	c.2007C>A**	p.D669E**	T/N	100/100	+	–
	0	1	19	c.2168A>G	p.H723R	14	c.1579A>C	p.T527P	N/N	115‡	–	–
	5	6	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	47.5/62.5	–	–
	1	2	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	105/93.75§	Unknown	Unknown
Midpoint enlargement	0	3	Intron 7	c.919-2A>G	Splice site mutation	17	c.2007C>A**	p.D669E**	T/N	82.5/93.75#	+	+
	0	8	19	c.2168A>G	p.H723R	18	c.2074T>C**	p.F692L**	N/N	75/115	+	+
	7	10	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	60/15	+	+
	0	35	10	c.1229C>T	p.T410M	17	c.1975G>C	p.V659L	N/N	97.5/87.5	+	+

TABLE II.
(*Continued*)

EVA Morphology	Age at Deafness Diagnosis, yr	Age, yr	Allele 1 Exon/Intron	Allele 1 DNA Change	Allele 1 Amino Acid Change or Splicing Mutation	Allele 2 Exon/Intron	Allele 2 DNA Change	Allele 2 Amino Acid Change or Splicing Mutation	T/N	Hearing Level, R/L, dBHL*	Fluctuation of Hearing	Progression of Hearing Loss
Borderline enlargement	0	5	Intron 7	c.919-2A>G	Splice site mutation	19	c.2168A>G	p.H723R	T/N	73.75/77.5	+	+
	2	2	Intron 14	c.1614+1G>A	Splice site mutation	10	c.1229C>T	p.T410M	T/N	55‡	Unknown	Unknown
	4	4	19	c.2168A>G	p.H723R	19	c.2168A>G	p.H723R	N/N	106.25/88.75#	Unknown	+
	0	6	14	c.1586T>G	p.I529S	19	c.2168A>G	p.H723R	N/N	80/66.25	–	–
	4	14	10	c.1229C>T	p.T410M	19	c.2168A>G	p.H723R	N/N	118.75/58.75	+	+

*Value without slash indicates binaural stimulus.
†Auditory brainstem response.
‡Conditioned Orienting Response.
**Candidate novel mutation.
§Auditory steady state response.
#Conditioned Play Audiometry.
EVA = enlarged vestibular aqueduct; L = left; N = nontruncating; R = right; T = truncating.

not affect distribution of subjects among different hearing levels (data not shown). Next, the relation between hearing fluctuation and EVA morphology was investigated in 28 subjects for whom relevant audiometric data were available (Fig. 4C). Hearing fluctuations were detected in 15 subjects (54%) in total, and no significant differences were noted in the incidence of hearing fluctuations between the subgroups of EVA morphology ($P = .209$). Lastly, the relation between progression of hearing loss and EVA morphology was analyzed in 29 subjects for whom relevant clinical data were available (Fig. 4D). Twenty subjects (69%) had progressive hearing loss in total, and the results showed no significant differences in the incidence of progressive hearing loss between the subgroups of EVA morphology ($P = .207$).

DISCUSSION

Although a variety of EVA criteria using the midpoint and aperture diameters of the vestibular aqueduct have been proposed to date,[1,6–10] our study is the first attempt to divide EVA into subgroups based on the shape and size of the vestibular aqueduct, and the first to investigate the possible relationship of these subgroups with genotypes and audiometric findings. *SLC26A4* mutations were detected in 72% of the Japanese subjects with bilateral EVA. Among these *SLC26A4* mutations, four mutations were novel. The discovery of these novel mutations would expand the *SLC26A4* mutation spectrum, thereby contributing to a more accurate gene-based diagnosis of hearing loss with EVA.

Nearly all subjects with aperture, aperture and midpoint, and midpoint enlargement presented *SLC26A4* mutations, suggesting that subjects with these EVA subgroups are most likely to be diagnosed with Pendred syndrome or DFNB4. Conversely, only approximately 30% of subjects with borderline enlargement had *SLC26A4* mutation, which suggests that the majority of subjects in this EVA subgroup have a pathological mechanism other than Pendred syndrome or DFNB4.

None of the 47 EVA subjects enrolled in the present study had only a single *SLC26A4* mutation allele. This finding is in striking contrast with previous research reporting single *SLC26A4* mutation alleles in approximately one third of Caucasian subjects with EVA.[3,4,20–22] This discrepancy might be associated with Japanese subjects, who were reported to have a spectrum of *SLC26A4* mutations distinct from that of Caucasian subjects.[22] One possible explanation is that the development of EVA in the Caucasian population may more frequently involve mutations in the introns or promoter regions of the *SLC26A4* than that in the Japanese population. Another possibility is that the Caucasian population may have higher mutation frequencies in genes than the Japanese population, causing digenic hearing loss in association with heterozygous *SLC26A4* mutations (e.g., *KCNJ10* and *FOXI1*).[23–25] The other possible explanation for the discrepancy is that the present study registered only subjects with bilateral EVA, whereas previous studies included those with unilateral hearing loss or unilateral EVA. This implicates the hypothesis that

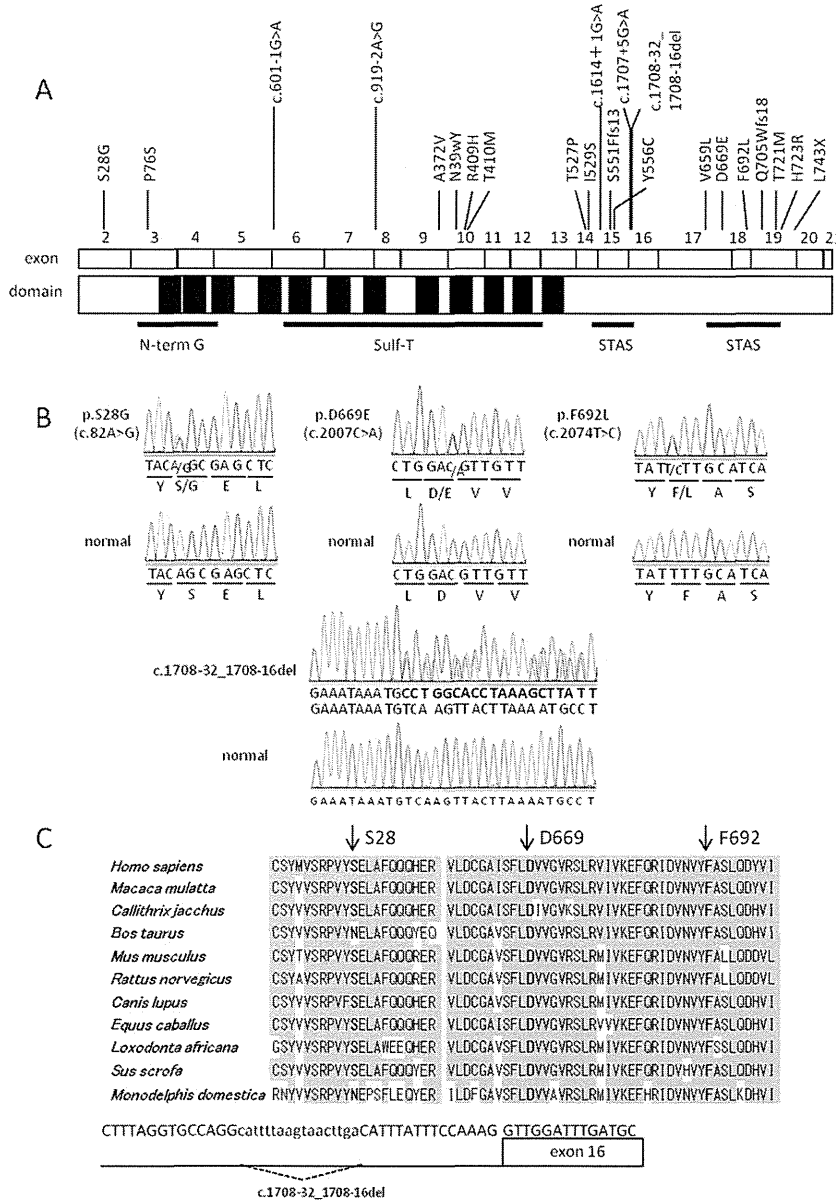

Fig. 3. The location of each mutation in *SLC26A4*, the evolutionary conservation of the amino acids, and nucleotides affected by the novel missense and splice site mutations. (A) Location of the *SLC26A4* mutations found in this study. Putative transmembrane regions are shown in black. N-term G = sulfate transporter N-terminal domain with Gly motif; STAS = sulfate transporter and anti–sigma factor antagonist domain; Sulf-T = sulfate transporter family domain. (B) electropherograms of the novel mutations and the corresponding sequence from normal alleles. Note that the nucleotide sequence of c.1708-32_1708-16del is shown reverse complementary. (C) Upper: multiple alignments of SLC26A4 protein orthologues at two noncontiguous regions. Arrows indicate affected amino acids. Conserved amino acids are shaded in gray. Lower: boundaries between intron 15 and exon 16 and deleted nucleotides are indicated at the bottom. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

biallelic mutations of *SLC26A4* are more strongly associated with bilateral EVA.

Our analysis of subjects with *SLC26A4* mutations revealed no significant difference in the proportion of truncating and nontruncating *SLC26A4* mutations between subgroups of EVA morphology. This suggests that, in addition to malfunction of the *SLC26A4* protein, environmental factors or genes other than *SLC26A4* may contribute to variations in vestibular aqueduct morphology.

Some researchers argue that there is no significant relationship between the degree of the EVA and the severity and progression of hearing loss and hearing fluctuations, whereas others propose that there is a significant relationship.[26] In the present study, no significant differences were detected in the level, fluctuation, and progression of hearing loss between the subgroups of EVA morphology, indicating that characteristics of hearing loss cannot be predicted based on the EVA morphology in subjects with Pendred syndrome or DFNB4.

CONCLUSION

Almost all the subjects with aperture, aperture and midpoint, and midpoint enlargement of EVA had two *SLC26A4* mutation alleles, whereas more than two thirds

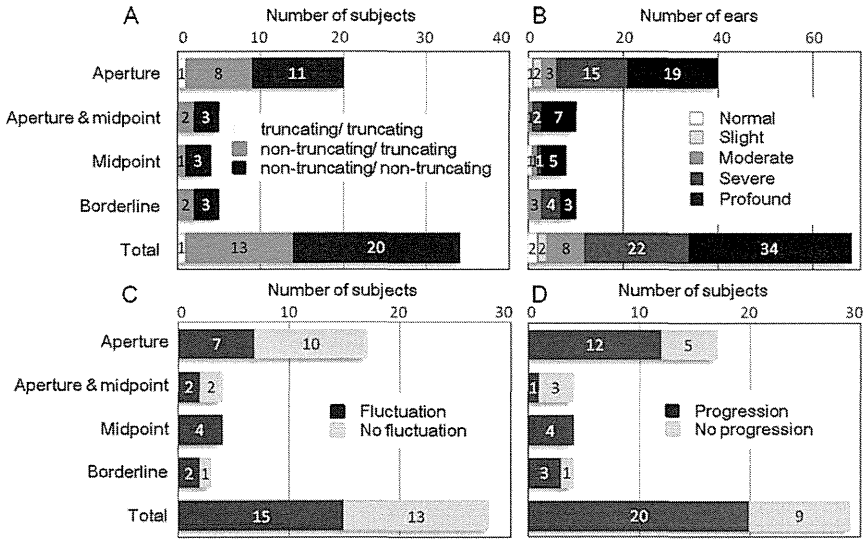

Fig. 4. Association of enlarged vestibular aqueduct (EVA) subgroups with *SLC26A4* genotypes or characteristics of hearing loss in subjects with biallelic *SLC26A4* mutations. (A) Proportion of *SLC26A4* genotypes in subjects of each EVA subgroup. (B) Proportion of different hearing levels in ears of each EVA subgroup. (C) Prevalence of fluctuating hearing loss in subjects of each EVA subgroup. (D) Prevalence of progressive hearing loss in subjects of each EVA subgroup.

of subjects with borderline enlargement of EVA had no *SLC26A4* mutation alleles. Analysis of subjects with two *SLC26A4* mutation alleles revealed no significant correlation between the morphologic subgroups of EVA and *SLC26A4* genotypes or characteristics of hearing loss, suggesting that the subgroups of EVA morphology may be associated with factors other than genotypes of *SLC26A4* mutations and that the subgroups of EVA morphology are not a predictive factor for characteristics of hearing loss.

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Short Communication

Chronic constipation recognized as a sign of a *SOX10* mutation in a patient with Waardenburg syndrome

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ABSTRACT

Waardenburg syndrome is characterized by hearing loss, pigmentation abnormalities, dysmorphologic features, and neurological phenotypes. Waardenburg syndrome consists of four distinct subtypes, and *SOX10* mutations have been identified in type II and type IV. Type IV differs from type II owing to the presence of Hirschsprung disease. We identified a de novo nonsense mutation in *SOX10* (p.G39X) in a female pediatric patient with Waardenburg syndrome with heterochromia iridis, profound bilateral sensorineural hearing loss, inner ear malformations, and overall hypopigmentation of the hair without dystopia canthorum. This patient has experienced chronic constipation since she was a neonate, but anorectal manometry showed a normal anorectal reflex. Chronic constipation in this patient was likely to be a consequence of a mild intestinal disorder owing to the *SOX10* mutation, and this patient was considered to have a clinical phenotype intermediate between type II and type IV of the syndrome. Chronic constipation may be recognized as indicative of a *SOX10* mutation in patients with Waardenburg syndrome.

1. Introduction

Waardenburg syndrome (WS) is a hereditary disease characterized by sensorineural hearing loss and pigmentation abnormalities that is classified into four subtypes according to clinical symptoms (Read and Newton, 1997). WS with dystopia canthorum (W index > 1.95) is classified as WS type I (WS1), WS without dystopia canthorum as type II (WS2), WS with symptoms of WS1 and musculoskeletal abnormalities in the upper extremities as type III (WS3), and WS with symptoms of WS2 and Hirschsprung disease as type IV (WS4). In addition, WS is genetically heterogeneous (Pingault et al., 2010). There are eight confirmed loci (viz., WS1/WS3 on 2q36.1, WS2A on 3p14.1-p12.3, WS2B on 1p21-p13.3, WS2C on 8p23, WS2D on 8q11.21, WS2E/WS4C on 22q13.1, WS4A on 13q22.3 and WS4B on 20q13.2-q13.3) and six known genes (viz., *PAX3* for WS1/WS3, *MITF* for WS2A, *SNAI2* for WS2D, *SOX10* for WS2E/WS4C, *EDNRB* for WS4A and *EDN3* for WS4B loci) for WS (Kapoor et al., 2012). Mutations in *SOX10* were first reported in WS4 and later in WS2 (Bondurand et al., 2007; Pingault et al., 1998). WS4 accompanied by neurological symptoms owing to insufficient myelination of central or peripheral nerves is called peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease (PCWH), and PCWH is also associated with *SOX10* mutations (Inoue et al., 1999). In addition, *SOX10* mutations have been identified in patients with WS2 with similar neurological symptoms (Barnett et al., 2009). Some patients with WS with *SOX10* mutations have intestinal pseudo-obstruction with symptoms of ileus despite the presence of enteric ganglia (Elmaleh-Bergès et al., 2012; Pingault et al., 2002).

In this study, we report a 3-year-old girl who presented with chronic constipation as well as hearing loss, inner ear malformations, and pigmentation abnormalities. Her condition was considered to be a clinical phenotype intermediate between type II and type IV of the syndrome, and suggested that chronic constipation may be a sign of a *SOX10* mutation in patients with WS.

2. Materials and methods

2.1. Clinical evaluation

All the procedures were approved by the Ethics Review Committee of National Tokyo Medical Center and Chiba Children's Hospital, and were carried out only after a written informed consent had been obtained from each individual or parents of the child.

Abbreviations: WS, Waardenburg syndrome; ABR, auditory brainstem response; PCWH, peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease; *CT*, computed tomography; NMD, nonsense-mediated mRNA decay.

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Hearing loss in the proband was assessed by auditory brainstem response (ABR) and play audiometry. Malformations of the inner ear were assessed by computed tomography (CT) of the temporal bone. During the recording sessions for ABR and CT, the patient was in a state of induced sleep with triclofos sodium (80 mg/kg, administered orally). ABR studies were obtained using the Neuropack device (Nihonkohden Corporation, Tokyo, Japan). Play audiometry was performed by an Audiometer AA-75 (Rion, Tokyo, Japan). Based on pure-tone air-conduction thresholds, the degree of hearing loss was determined by the better ear pure-tone average across the frequencies 0.5, 1, 2, and 4 kHz, and it was classified as mild (20–40 dB), moderate (41–70 dB), severe (71–95 dB), or profound (>95 dB) according to the recommendations for the description of audiological data by the Hereditary Hearing Loss Homepage (http://hereditaryhearingloss.org). CT was conducted using a LightSpeed VCT 64-slice scanner (GE Healthcare, CT, USA). Anorectal reflex was measured by a Anorectal reflex monitor Pocket monitor (Star Medical, Tokyo, Japan).

2.2. Gene analysis

Genomic DNA was extracted from blood samples using the Gentra Puregene Blood kit (QIAGEN, Venlo, Netherlands), and primers specific for *SOX10* (GenBank NG_007948.1) (http://www.ncbi.nlm.nih.gov/Genbank/) were designed. For PCR amplification of *SOX10* exons 1, 2 and 3, the primer sets 5′-TGTAAAACGACGGCCAGTagatgggtttagctggagca-3′, and 5′-CAGGAAACAGCTATGACCaatccacccgaagctagagg-3′, and 5′-TGTAAAACGACGGCCAGTtctcaccctccagcccatga-3′, and 5′-CAGGAAACAGCTATGACCtgccatccagccatctcctg-3′, and 5′-TGTAAAACGACGGCCAGTcccgactcatgctgccaaa-3′, and 5′-CAGGAAACAGCTATGACCcccgacctgtcagcctcttca-3′ were used with the PC-818 Program Temp Control System (Advanced Science and Technology Enterprise Corporation, Tokyo, Japan). Each primer is specific for a given genomic sequence (lower case) and was used in combination with either a universal forward M13 (upper case) or reverse M13pUC (upper case) primer. The following PCR program was used: 98 °C for 5 min; 35 cycles of 98 °C for 10 s, 62 °C for 10 s, and 72 °C for 1.2 min; and then 72 °C for 3 min. PrimeSTAR HS DNA polymerase (Takara Bio, Shiga, Japan) was used for the PCR. The amplicons were sequenced using the ABI 3730 DNA sequence analyzer with the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA).

3. Results

3.1. Clinical features

A 3-year-old girl born to a Japanese mother and a Chinese father was referred to our hospital with the chief complaint of unstable neck at the age of 4 months. She did not have any prenatal abnormalities. Her irises were light blue and her hair was mostly brown. When hearing acuity was tested by ABR, the wave V threshold was 105dBnHL for the right ear, and the left ear did not respond to the sound of 105dBnHL. No dystopia canthorum was observed. Based on these clinical features, she was first suspected to have WS2. No other members of her family had similar symptoms (Fig. 1). Play audiometry at 2 years and 10 months of age revealed bilateral profound sensorineural hearing loss (Fig. 2). CT of the temporal bone revealed hypoplastic cochleae in which the first turn and the second turn were not separated (Figs. 3A, B, arrows), and the cochlear nerve canal was intercepted by a bony plate (Figs. 3C, D, arrows) in both ears. The vestibule was enlarged in both ears (Figs. 3E, F). Arches of the superior and lateral semicircular canals were also enlarged near the vestibule. The posterior semicircular canal was absent, and potential anlagen of the posterior semicircular canal were visible in both ears (Figs. 3E, F; arrows). The vestibular aqueduct was normal in both ears.

Meconium passage was noted within 24 h of birth, but chronic constipation subsequently occurred. The patient did not experience a natural bowel movement for periods of 5 days or longer, and thus stimulant laxatives were administered to the patient daily. Anorectal manometry confirmed a normal anorectal reflex, indicating the presence of enteric ganglia (Fig. 4). No neurological symptoms indicative of neuropathy were noted.

3.2. Genetic features

Given the patient's clinical manifestations, she was suspected of having WS2 or WS4. Thus, *MITF* and *SOX10* were initially analyzed. The DNA of the patient and particular family members (Fig. 1) were extracted from venous blood, and all *MITF* and *SOX10* coding exons and their flanking intronic sequences were amplified using polymerase chain reaction (PCR; primer sequences and PCR conditions are available upon request). DNA sequencing of these PCR products did not reveal any mutations in *MITF*, but a heterozygous *SOX10* mutation of c. 115G>T

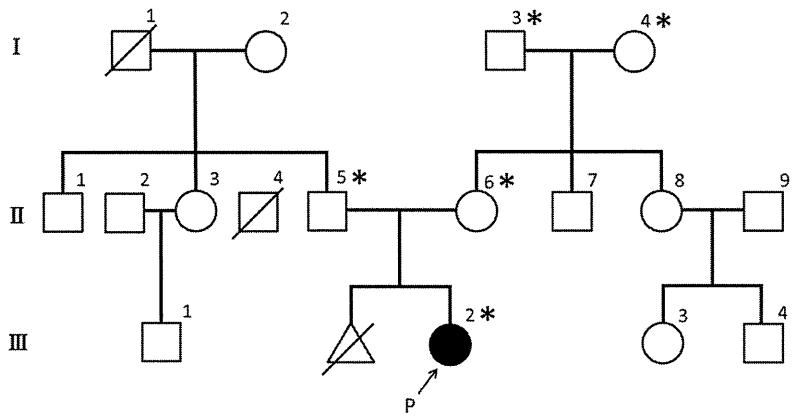

Fig. 1. Family pedigree of the family. Individuals who were examined and whose blood samples were collected for DNA analysis are indicated by asterisks. Only the proband (P) had the characteristic symptoms indicating WS, and no other familial members had similar symptoms. The pedigree was described in the style that followed "Recommendations for standardized human pedigree nomenclature" (Bennett et al., 1995). Roman numerals indicate generations of the family and individuals are numbered for identification by Arabic numerals in each generation. Open squares and open circles indicate unaffected males and unaffected females, respectively. Crossed squares and a crossed triangle represent deceased males and an abortion, respectively. A filled circle indicates a female affected with WS.

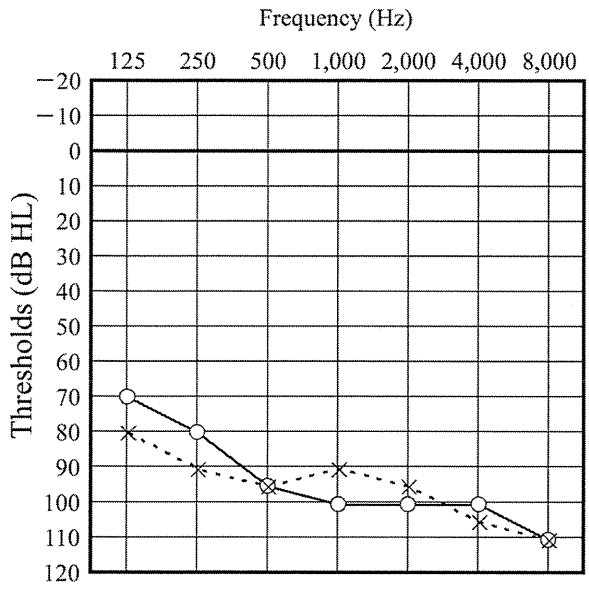

Fig. 2. Play audiometry of the patient. Pure tone thresholds of the right ear are indicated with "○", and those of the left ear are indicated with "×". It was shown that the patient had bilateral profound hearing loss according to the definitions of the degree of hearing loss described in the Materials and methods.

(p.G39X) was identified in the patient (Fig. 5). This mutation was not found in the parents, maternal grandparents, or the control group which consisted of 96 unrelated Japanese individuals with normal hearing as determined with pure tone audiometry.

4. Discussion

The present patient had WS with a confirmed de novo *SOX10* nonsense mutation and presented with chronic constipation. Although Hirschsprung disease and intestinal pseudo-obstruction have been reported in association with WS4, the chronic constipation observed in this patient did not fit these profiles. There have been two reported patients who presented constipation in WS associated with *SOX10* mutations (Chaoui et al., 2011). However, unlike the patient in the present study, those two patients had clinical phenotype of peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, and WS or PCWH which are associated with peripheral demyelinating neuropathy and central dysmyelinating leukodystrophy and such neurological phenotypes suggest *SOX10* mutations by themselves without constipation. In addition, constipation in the two patients could be caused by central dysmyelinating leukodystrophy. Therefore, their constipation may not be caused by the abnormality in enteric ganglia due to *SOX10* mutations.

On the other hand, the present patient was absent from the neurological phenotypes, and considered as having a clinical phenotype intermediate between WS2 and WS4. Because WS2 and WS4 have several causative genes including *MITF*, *SOX10*, *EDN3*, *EDNRB*, and *SNAI2*, constipation was the only phenotype suggesting *SOX10* mutations among those candidate

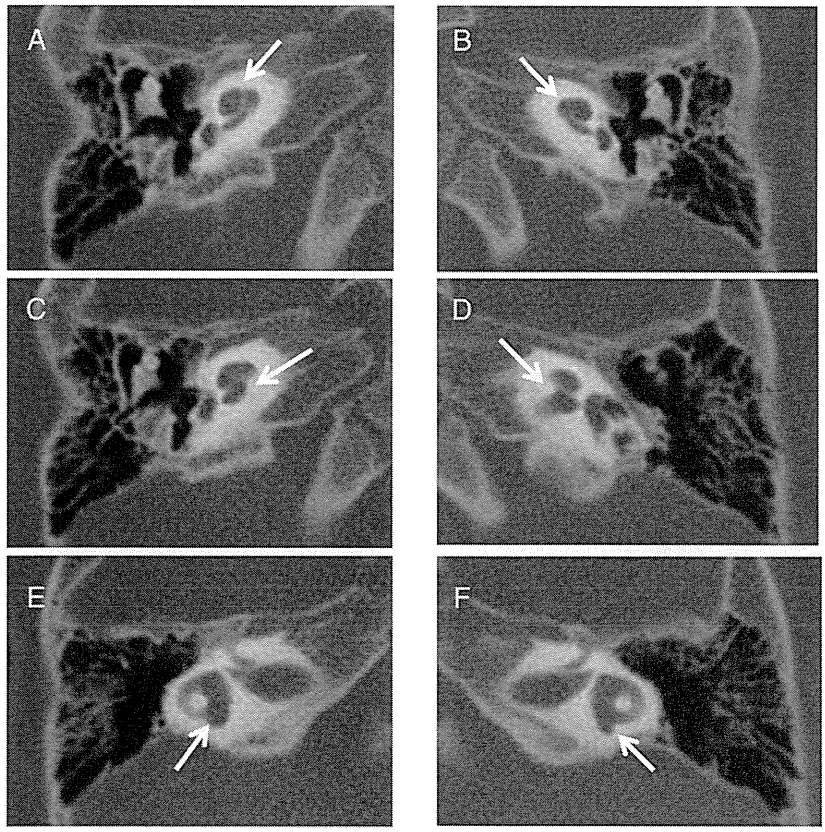

Fig. 3. CT of the temporal bone of the patient. The right ear is shown in the left column (A, C, E), and the left ear is shown in the right column (B, D, F). Each row shows the same level of horizontal section of the inner ear. Bilateral inner ear malformations of this patient are shown in these images. Hypoplasia of the bilateral cochleae (A, B; arrows). Atresia of the bilateral cochlear nerve canals (C, D; arrows). Enlarged vestibules with potential anlagen of the posterior semicircular canals (E, F; arrows).

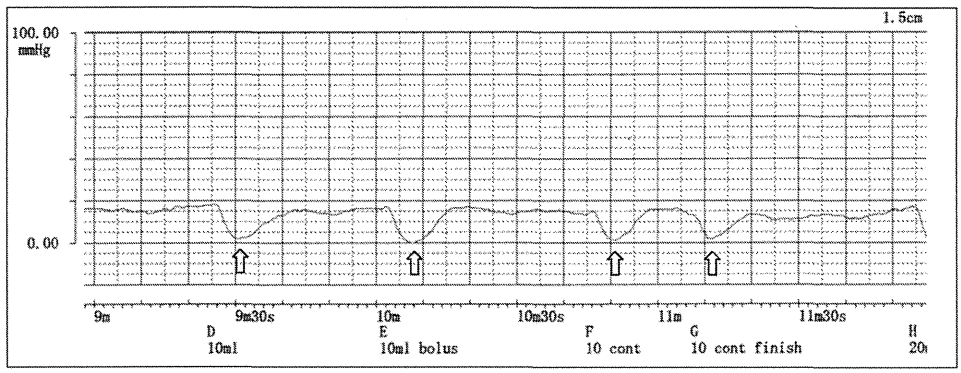

Fig. 4. Anorectal manometry of the patient. Anorectal pressure plots along the perpendicular axis and time course plots along the horizontal axis. A normal anorectal reflex (indicated with upward-pointing arrows) was confirmed repeatedly in this patient.

genes in the present patient. In addition, because the present patient did not have the neurological phenotypes, constipation in this patient was likely to be caused by abnormality in enteric ganglia, which suggested

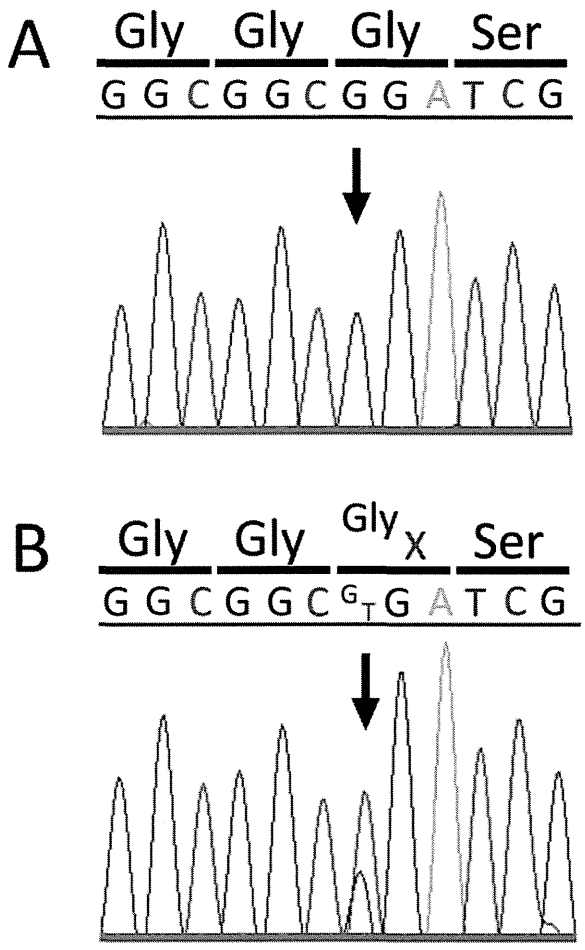

Fig. 5. Sequence chromatogram of *SOX10*. A: In the control, a homozygous G indicated by an arrow is the first nucleotide of the codon 39 for glycine (Gly). B: In the proband, a heterozygous G to T transition (arrow) at the same position leads to replacement of glycine (Gly) at the codon 39 with a stop codon (X), which causes premature termination of protein synthesis.

SOX10 mutations. Thus, the present patient demonstrated additional clinical significance of constipation in WS, and suggested that chronic constipation may be an important clinical sign of *SOX10* mutations in patients with WS, especially those without the neurological phenotypes.

Sham et al. (2001) examined the relationship between *SOX10* mutation sites and the severity of Hirschsprung disease by analyzing patients reported in the literature as well as their own patients. Patients with nonsense mutations in upstream regions of *SOX10* had aganglionosis only in short regions of the intestine that manifested as mild Hirschsprung disease, whereas patients with nonsense mutations in the last exon had aganglionosis along the entire colon and severe Hirschsprung disease. According to Inoue et al. (2004), in diseases with a dominant inheritance pattern, when a nonsense mutation is located 50 bases or more upstream of the last exon–exon junction, nonsense-mediated mRNA decay (NMD) occurs and causes haploinsufficiency, leading to a mild phenotype. In contrast, a nonsense mutation located in a region where NMD does not occur induces a dominant-negative effect and results in a severe phenotype. The p.G39X mutation in this patient was the most upstream nonsense mutation among those reported to date. Therefore, the mild phenotype in this patient is likely due to haploinsufficiency caused by NMD.

The other reported patients with the mutation in the same region of *SOX10* also presented with mild intestinal features (Pingault et al., 2002; Sham et al., 2001). A patient carried a nonsense mutation (p.R43X), and another patient carried a frameshift mutation (p.E57SfsX52). Both patients were affected with Hirschsprung disease, and found to have aganglionosis in a short segment of the intestine. We hypothesize that intestinal disorders owing to *SOX10* mutations can be viewed as a continuum: (1) with severe effects, the absence of enteric ganglion cells leads to severe Hirschsprung disease, especially with aganglionosis in long segment; (2) with moderate effects, Hirschsprung disease becomes mild type with aganglionosis in short segment, or, enteric ganglion cells are present, but intestinal pseudo-obstruction develops and causes symptoms similar to those of Hirschsprung disease; and (3) with mild effects, enteric ganglion cells are present and only chronic constipation may occur. Chronic constipation, which is a common clinical symptom that can easily be overlooked when it is mild, may be indicative of a *SOX10* mutation in patients with WS.

The present patient also had inner ear malformations. WS can be accompanied by inner ear malformations in some cases (Madden et al., 2003; Oysu et al., 2001). A recent study found inner ear malformations in patients with WS2, WS4, and PCWH with confirmed *SOX10* mutations (Elmaleh-Bergès et al., 2012). According to this study, all 15 patients examined had absent, abnormally shaped, or abnormally sized bilateral semicircular canals. No abnormalities were observed in the cochleae of 13 of the 15 patients. Because abnormalities were

found in the lateral semicircular canal in 100% of these patients, in the posterior semicircular canal in 93%, and in the superior semicircular canal in 87%, bilateral malformations in semicircular canals are considered to be indicative of WS that is associated with *SOX10* mutations. The patient in the present study was consistent with the proposed relationship between the inner ear malformation and *SOX10* mutations.

Conflict of interest

None.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Severe Congenital Lipodystrophy and a Progeroid Appearance: Mutation in the Penultimate Exon of *FBN1* Causing a Recognizable Phenotype

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Recently, three marfanoid patients with congenital lipodystrophy and a neonatal progeroid appearance were reported. Although their phenotype was distinct from that of classic Marfan syndrome, they all had a truncating mutation in the penultimate exon, i.e., exon 64, of *FBN1*, the causative gene for Marfan syndrome. These patients might represent a new entity, but the exact phenotypic and genotypic spectrum remains unknown. Here, we report on a girl born prematurely who exhibited severe congenital lipodystrophy and a neonatal progeroid appearance. The patient exhibited a characteristic growth pattern consisting of an accelerated growth in height with a discrepant poor weight gain. She had a characteristic facial appearance with craniosynostosis. A mutation analysis identified c.8175_8182del8bp, p.Arg2726Glufs*9 in exon 64 of the *FBN1* gene. A review of similar, recently reported patients revealed that the cardinal features of these patients include (1) congenital lipodystrophy, (2) premature birth with an accelerated linear growth disproportionate to the weight gain, and (3) a progeroid appearance with distinct facial features. Lines of molecular evidence suggested that this new progeroid syndrome represents a neomorphic phenotype caused by truncated transcripts with an extremely charged protein motif that escapes from nonsense-mediated mRNA decay, altering FBN1-TGF beta signaling, rather than representing the severe end of the hypomorphic phenotype of the FBN1-TGF beta disorder spectrum. We propose that this marfanoid entity comprised of congenital lipodystrophy, a neonatal progeroid appearance, and a peculiar growth profile and caused by rare mutations in the penultimate exon of *FBN1*, be newly referred to as marfanoid–progeroid syndrome. © 2013 Wiley Periodicals, Inc.

Key words: congenital lipodystrophy; progeroid appearance; Marfan syndrome

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INTRODUCTION

Recently, Graul-Neumann et al. [2010] reported a distinctive marfanoid patient with severe congenital lipodystrophy and a progeroid appearance arising from a truncating mutation in the penultimate exon, i.e., exon 64, of *Fibrillin (FBN)1* on 15q21.1, the causative gene for Marfan syndrome (OMIM 154700) [Graul-Neumann et al., 2010]. The documentation of two patients with a similar phenotype and mutation in the penultimate exon of the *FBN1* gene suggested that this *FBN1*-related progeroid syndrome represents a new disease entity [Goldblatt et al., 2011; Horn and Robinson, 2011]. These three patients presented with a progeroid appearance and arachnodactyly at birth. Their extreme thinness was distinctive from so-called neonatal Marfan syndrome, and none of the three patients had been diagnosed as such. All three patients were suspected of having an *FBN1* mutation based on the

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Abbreviations: FBN, fibrillin; NMD, nonsense-mediated mRNA decay; TGF, transforming growth factor.
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emergence of some features of Marfan syndrome, including accelerated growth and subluxation of the lens, during adolescence. However, the striking progeroid appearance precluded a clinical diagnosis of Marfan syndrome. Two clinical questions remain to be elucidated: (1) what is the range of *FBN1* mutations that can be associated with this distinctive phenotype, and (2) what is the molecular basis of the phenotypic difference between classic Marfan syndrome and this presumably new disease entity.

Here, we document a young girl who had a distinctive neonatal progeroid presentation and a heterozygous mutation in exon 64 of *FBN1*. She had a previously undescribed critical feature, craniosynostosis, which is the hallmark of Shprintzen-Goldberg syndrome (OMIM182212) [Carmignac et al., 2012]. The documentation of craniosynostosis in this progeroid syndrome illustrates a phenotypic overlap among FBN1-transforming growth factor (TGF) beta signaling pathway disorders.

CLINICAL REPORT

The propositus was a Japanese girl born to non-consanguineous parents who did not have any family history of inherited conditions. The pregnancy was complicated by intrauterine growth retardation and oligohydramnios. The propositus was born at 34 and 3/7 weeks of gestation via emergency cesarean section for fetal tachycardia. The Apgar scores were 8 and 9 at 1 and 5 min, respectively. Her birth weight was 1,427 g (−2.3 SD), her length was 40 cm (−1.8 SD), and her head circumference was 30.6 cm (−0.3 SD). A physical examination at birth revealed a progeroid appearance, wide-open anterior fontanelle, low-set ears, long arms and legs, arachnodactyly, and arthrogryposis, especially in the lower extremities. During her hospital stay, she developed jaundice, for which she underwent phototherapy. She was noted to have a transient elevated blood pressure. A renal ultrasound showed mild right hydronephrosis with no evidence of vesico-ureteral reflex on a voiding cystogram. She continued to take lisinopril until the age of 2 years, when her blood pressure normalized.

During her infancy and childhood, she consistently had a poor weight gain, with a weight of 5.9 kg (−2.8 SD) at 1 year, 11.7 kg (−1.8 SD) at 4 years and 2 months, and 21.7 kg (−1.6 SD) at 10 years of age. There was a disproportionately accelerated height growth that is 70.9 cm (−0.8 SD) at 1 year, 107.6 cm (+1.5 SD) at 4 years and 2 months, and 148.9 cm (+1.9 SD) at 10 years of age (Fig. 1). As for her psychomotor development, she met all the developmental milestones without any delay and she attended a regular school. Standard blinded neuropsychological testing using the Japanese version of the Stanford–Binet test, i.e., the Tanaka–Binet test, indicated an intelligent quotient of 122 at the age of 5 years and 7 months. The presence of craniosynostosis and marfanoid features raised a clinical suspicion of Loeys–Dietz syndrome but an analysis of the *TGFBR1* and *TGFBR2* genes did not reveal any pathologic mutations.

On examination at 10 years of age, she was extremely thin with little palpable subcutaneous adipose tissue. Her body mass index was 9.8 kg/m^2 (−7.3 SD) [Inokuchi et al., 2007]. She had arachnodactyly, a progeroid appearance and scaphocephaly with a prominent forehead, proptosis, and pectus excavatum. Since she complained of a recent decline in her visual acuity, she underwent a detailed ophthalmologic examination that revealed bilateral severe myopia, and proptosis. There was no subluxation of the lenses. The optic discs were enlarged.

Imaging studies of her entire neuroaxis revealed craniosynostosis of the posterior portion of the sagittal suture (Fig. 2), mild enlargement of the ventricles (consistent with arrested hydrocephalus), and lumbosacral dural ectasia. An echocardiogram showed no enlargement of the sinus of Valsalva, allowing annuloaortic ectasia to be ruled out.

MOLECULAR ANALYSIS

Genomic DNA was extracted from a whole blood sample of the propositus. A mutation analysis panel (SureSelect XT-Auto custom; Agilent Technologies, Santa Clara, CA) was custom-designed to include most of the causative genes listed in the classic textbook of dysmorphology: *Smith's Recognizable Patterns of Human Malformation* [Jones, 2006] (the list of genes is available upon request). Sequencing of the propositus' PCR products using this custom-designed mutation analysis panel and a next-generation sequencer (MiSeq; Illumina, Inc., San Diego, CA) enabled a heterozygous 8 bp deletion to be identified, extending from nucleotides 8,175–8,182 relative to the adenine of the start codon in exon 64 of the *FBN1* gene that is c.8175_8182del8bp p.Arg2726Glufs*9. This mutation was predicted to result in a frame-shift truncation of the transcript encoded by exon 64 of *FBN1*. Sanger sequencing of the same PCR product amplified from exon 64 and flanking introns of *FBN1* with primer (forward: 5′-tcacaactgcaaggaacagg-3′, reverse: 5′-cttggaggaaaccacaggaa-3′) confirmed the heterozygous 8-bp deletion in exon 64 of *FBN1* gene.

DISCUSSION

Here, we document a patient with a heterozygous mutation in exon 64 of the *FBN1* gene with neonatal progeroid presentation. Her presentation was distinct from classic Marfan syndrome or neonatal Marfan syndrome, both of which are caused by an *FBN1* mutation. We concluded that this constellation of phenotypes represents a clinically recognizable entity with a common molecular basis.

We reviewed the detailed clinical characteristics of the four patients, including the propositus, in a tabular form (Table I). Three distinct clinical features were present in all the patients: (1) congenital lipodystrophy, (2) characteristic growth patterns with prematurity (range: 28–36 weeks of gestation), accelerated height growth that cross growth chart channels and a discrepant poor weight gain, and (3) a progeroid appearance with characteristic facial features including proptosis, downslanting palpebral fissures, and retrognathia. Mandatory features that overlap with those of Marfan syndrome included arachnodactyly, digital hyperextensibility, myopia, dural ectasia, and normal psychomotor development. We propose that these signs should represent the clinical diagnostic criteria for this condition, for which we propose to refer to as marfanoid–progeroid syndrome.

Apart from severe congenital lipodystrophy and a progeroid appearance, the presence of craniosynostosis in the context of marfanoid features overlaps with Shprintzen–Goldberg syndrome.

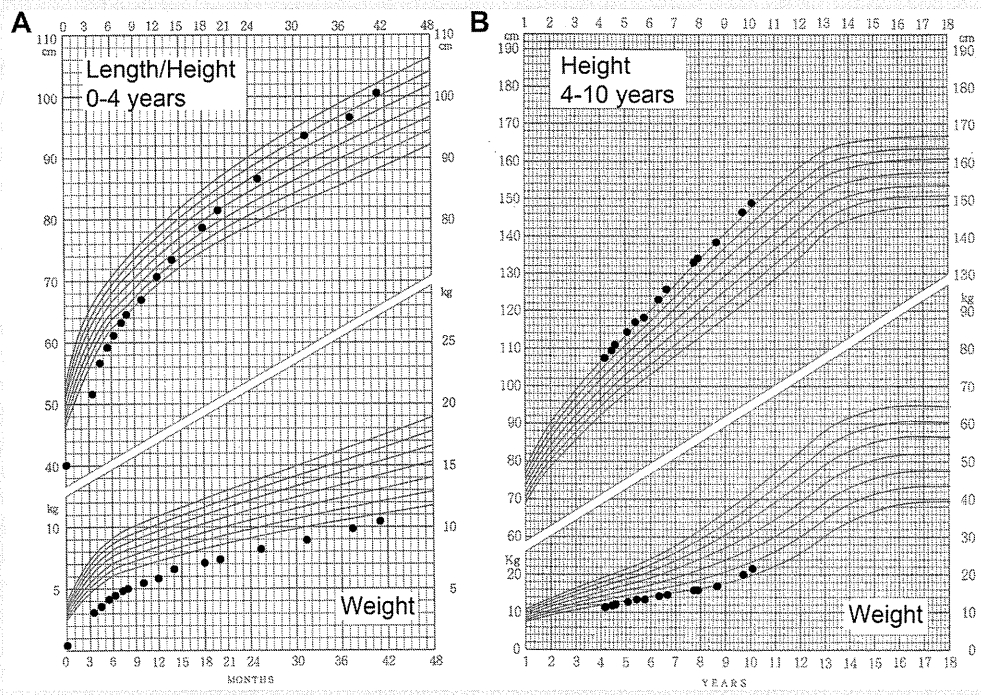

FIG. 1. Accelerated linear growth disproportionate to weight gain during childhood. Channels on the growth charts represent 3rd, 10th, 25th, 50th, 75th, 90th, and 97th centiles on the Japanese standard physical growth chart [Tsuzaki et al., 1987]. Note that the growth curve from birth to 48 months of age (A) shows a disproportionately accelerated height growth that crosses growth chart channels. The growth curve from 4 to 10 years of age (B) shows a poor weight gain that persisted throughout adolescence.

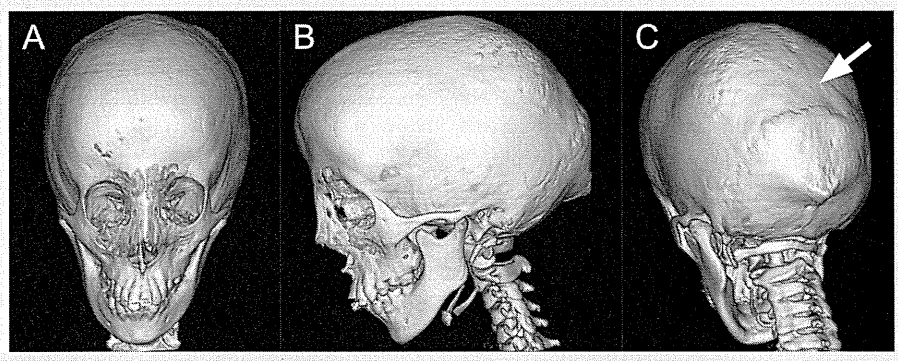

FIG. 2. Cranial computed tomography with three-dimensional reconstruction. Note the prominent forehead and marked scaphocephaly on the frontal view (A) and lateral view (B). A partial craniosynostosis of the posterior sagittal suture is visible (arrow, C).

This autosomal dominant disorder is caused by mutations in *SKI*, a repressor of the TGF beta signaling pathway, and is comprised of craniosynostosis, craniofacial abnormalities, and marfanoid features [Kosaki et al., 2006; Carmignac et al., 2012; Doyle et al., 2012]. It is notable that some patients with Loeys–Dietz syndrome (OMIM 609192), which is caused by a *TGFBR1* or *TGFBR2* mutation, can also present with craniosynostosis [Loeys et al., 2006]. The observation of craniosynostosis in these two classic syndromes and in the

TABLE I. Summary of the Patients With Severe Lipodystrophy and Progeroid Appearance and Mutations in Exon 64 of *FBN1*

	Patient 1, Graul-Neumann et al. [2010]	Patient 2, Goldblatt et al. [2011]	Patient 3, Horn and Robinson [2011]	Patient 4, the propositus	Phenotypic range among Patients 1–4	MFS[a]	SGS[a]	LDS[b]
Age reported/sex	25-year-old/female	20-year-old/male	3.5-year-old/female	10-year-old/female				
Gestational age	36 weeks	28 weeks	32 weeks	34 weeks	Range: 28–36 weeks			
Birth weight	1,780 g (−2.31 SD)	1.04 kg	1,185 g (−1.5 SD)	1,427 g (−2.3 SD)	Below −1.5 SD			
Mutation	c.8155_8156delAA	c.8156_8175del	c.8226+1G>T (IVS64+1G>T)	c.8175_8182del8bp	Exon/intron 64 of FBN1	*FBN1*	*SKI*	*TGFBR1/2*
Amino acids	p.Lys2719AspfsX18	p.Lys2719ThrfsX12	Splice site	p.Arg2726Glufs*9	"ETEKHKRN" motif at carboxyl terminus			
Congenital lipodystrophy	Present	Present	Present	Present	4/4	−	−	−
Extreme thinness (body mass index)	Present (13.3 kg/m^2 at 25-year-old)	Present (NR)	Present (12.4 kg/m^2 at 2-year-old)	Present (9.8 kg/m^2 at 10-year-old)	4/4	+	−	−
Arachnodactyly	Present	Present	Present	Present	4/4	+	+	+
Digital hyper-extensibility	Present	Present	NR	Present	3/4	+	+	+
Progeroid appearance	Present	Present	Present	Present	4/4	−	−	−
Retrognathia	Present	Present	Present	Present	4/4	−	+	+
Prominent forehead/scaphocephaly	NR	NR	Present	Present	2/4	−	+	−
Downslanting palpebral fissures	NR	Present	Present	Present	3/4	+	+	−
Craniosynostosis	NR	NR	NR	Present	1/4	−	+	+
Ectopia lentis	Present	Present	Absent	Absent	3/4	+	−	+
Proptosis	Present	Present	Present	Present	4/4	−	+	−
Severe myopia	Present	Present	Absent	Present	3/4	+	−	−
Cardiovascular complications	Dilatation of the aortic bulb and mild mitral prolapse	Absent	Mild mitral valve prolapse	Absent	2/4	+	−	+
Normal psychomotor development	Present	Present	Present	Present	4/4	+	−	+
Arrested hydrocephalus	NR	Present	Present	Present	3/4	−	+	−
Dural ectasia	Present	NR	NR	Present	2/4	+	−	−
Renal/genitourinary complications	NR	Absent	NR	Hydronephrosis	1/4	−	−	−
Hypertension	NR	NR	NR	Present	1/4	−	−	−

LDS, Loeys–Dietz syndrome; MFS, Marfan syndrome; SGS, Shprintzen–Goldberg syndrome; NR, not recorded Presence/absence of each phenotype was based on descriptions in [a]Jones [2006] and [b]Loeys et al. [2006].

propositus with the presumably new disease entity suggests that craniosynostosis might be a phenotypic component extending across various disorders that is caused by an aberrant FBN1-TGF beta signaling cascade.

Congenital lipodystrophy and a progeroid appearance are distinctive phenotypes among FBN1-TGF beta signaling disorders. However, it remains unclear whether these additional phenotypes represent a neomorphic mutation or the most severe end of the FBN1-TGF beta signaling spectrum. From a molecular standpoint, we tend to think that they represent a neomorphic mutation. The four progeroid patients had mutations in exon 64 of *FBN1*. Although no apparent genotype–phenotype relationship is known to exist between *FBN1* mutations and Marfan syndrome phenotypes, except for neonatal Marfan syndrome [Kainulainen et al., 1994], a clear genotype–phenotype relationship seems to exist in the four progeroid patients. One hypothetical mechanism would be a defective nonsense-mediated mRNA decay (NMD) triggered by a truncating mutation involving the most carboxyl terminus of the *FBN1* gene. In general, transcripts containing a premature termination codon trigger NMD, leading to mRNA decay or degradation. An exception to this rule is that transcripts with premature termination codons in the last exon and 50–55 bp of the penultimate exon are translated into truncated proteins [Khajavi et al., 2006]. Indeed, the four progeroid patients all had mutations near the 3′ end of the penultimate exon, i.e., exon 64, and their protein products likely escaped from NMD (Fig. 3). Moreover, the functional domain sequence encoded by exons 44–49 of *FBN1*, which releases endogenous TGF beta and stimulates downstream TGF beta receptor signaling [Chaudhry et al., 2007], would have been preserved in the four progeroid patients.

Although this hypothetical explanation seems plausible for the four progeroid patients with a mutation in exon 64, NMD fails to explain other patients with truncating mutations in exon 64 or 65 of *FBN1* who did not present with a progeroid appearance or severe lipodystrophy [Collod-Beroud et al., 1998; Palz et al., 2000; Rommel et al., 2005]. Hence, an additional mechanism in conjunction with NMD is needed to explain the presumed genotype–phenotype relationship by which the penultimate exon causes this specific phenotype. The presence of the "ETEKHKRN" protein motif in the carboxyl termini of the truncated transcripts may be associated with this unique phenotype (Fig. 3). Goldblatt et al. [2011] pointed out that the presence of an extremely charged protein sequence in the carboxyl terminus of the truncated transcripts may affect downstream protein–protein interactions. The observation that the above-mentioned patients with frame-shift mutations in exons 64–65 without progeroid phenotype lacked this "ETEKHKRN" protein motif further augments our hypothesis. These lines of evidence points to the interpretation that this new progeroid syndrome represents a neomorphic phenotype caused by truncated transcripts with an extremely charged protein motif that escape

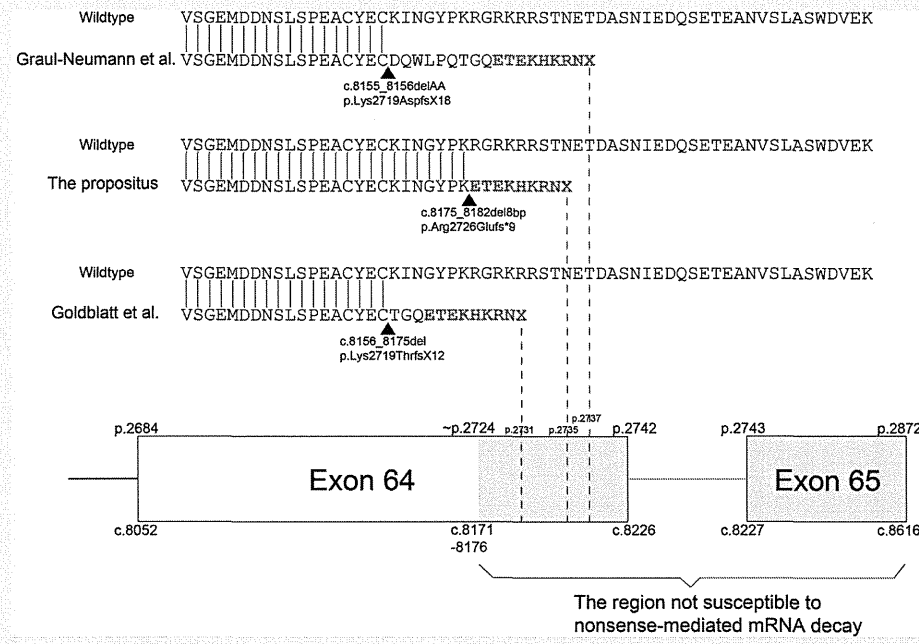

FIG. 3. Common aberrant motif caused by frame-shift mutations in progeroid patients. Predicted protein sequences are shown for the patients reported by Graul-Neurmann et al., Goldblatt et al. and the propositus (wildtype, above; patient, below). Note that all three patients had the same specific protein motif, "ETEKHKRN (in red)," at the carboxyl termini of the transcripts. The black triangles indicate the relative positions of the mutations. The dotted lines indicate the relative positions of the protein truncation on exon 64. The gray area in exons 64 and 65 indicates the genetic region where the truncating mutations are assumed not to trigger nonsense-mediated mRNA decay.

from nonsense-mediated mRNA decay, thereby altering FBN1-TGF beta signaling, rather than the severe end of the phenotypic spectrum of the FBN1-TGF beta signaling disorders.

In conclusion, marfanoid–progeroid syndrome should be included in the differential diagnosis of progeroid syndromes. The diagnostic clues to this new entity are a progeroid appearance accompanied by characteristic growth patterns: premature birth with accelerated height growth that cross growth chart channels and a discrepant poor weight gain, resulting in a severely reduced body mass index. This phenotypic combination should prompt a mutation analysis of the penultimate exon of *FBN1*.

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Mutations in *SERPINB7*, Encoding a Member of the Serine Protease Inhibitor Superfamily, Cause Nagashima-type Palmoplantar Keratosis

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"Nagashima-type" palmoplantar keratosis (NPPK) is an autosomal recessive nonsyndromic diffuse palmoplantar keratosis characterized by well-demarcated diffuse hyperkeratosis with redness, expanding on to the dorsal surfaces of the palms and feet and the Achilles tendon area. Hyperkeratosis in NPPK is mild and nonprogressive, differentiating NPPK clinically from Mal de Meleda. We performed whole-exome and/or Sanger sequencing analyses of 13 unrelated NPPK individuals and identified biallelic putative loss-of-function mutations in *SERPINB7*, which encodes a cytoplasmic member of the serine protease inhibitor superfamily. We identified a major causative mutation of c.796C>T (p.Arg266*) as a founder mutation in Japanese and Chinese populations. SERPINB7 was specifically present in the cytoplasm of the stratum granulosum and the stratum corneum (SC) of the epidermis. All of the identified mutants are predicted to cause premature termination upstream of the reactive site, which inhibits the proteases, suggesting a complete loss of the protease inhibitory activity of SERPINB7 in NPPK skin. On exposure of NPPK lesional skin to water, we observed a whitish spongy change in the SC, suggesting enhanced water permeation into the SC due to overactivation of proteases and a resultant loss of integrity of the SC structure. These findings provide an important framework for developing pathogenesis-based therapies for NPPK.

The congenital palmoplantar keratoses (PPKs) are a heterogeneous group of diseases. Phenotypic classification of hereditary PPKs is based mainly on the specific morphology and distribution of the hyperkeratosis, the presence or absence of associated features, and the inheritance pattern and is assisted by additional criteria such as the presence of skin lesions in areas other than the palms and soles, the age at onset of the hyperkeratosis, the severity of the disease process, and histopathological findings.[1]

"Keratosis palmoplantaris Nagashima"[2] or "Nagashima-type" PPK (NPPK)[3] has been proposed as a clinical entity included within the diffuse hereditary PPKs without associated features.[1] A familial case of two siblings was first reported as a distinct clinical type of PPK in 1989.[2,4] Because Nagashima briefly described this type of hereditary PPK in the Japanese literature in 1977,[5] the name "keratosis palmoplantaris Nagashima" was proposed.[2] Although about 20 cases of Japanese individuals with NPPK have been reported in the Japanese literature since then, this clinical entity was not described in detail in the English language literature until 2008.[3]

An autosomal recessive trait has been suggested in NPPK.[2,3] The clinical features of NPPK are characterized by well-demarcated reddish and diffuse palmoplantar hyperkeratosis that extends to the dorsal surfaces of the hands, feet, inner wrists, ankles, and the Achilles tendon area.[2–6] Involvement of the elbows and knees and high frequencies of hyperhidrosis on palms and soles have been noted.[3] Clinical observations revealed no differences between males and females, no seasonal change, and no association with squamous cell carcinoma or any other malignancy. Although mild T cell infiltration in the affected skin area has been reported,[7] the pathophysiology of the skin redness and hyperkeratosis are still uncharacterized.

An autosomal recessive trait, transgressive diffuse hyperkeratosis, and the absence of associated features are also characteristic of Mal de Meleda (MDM [MIM 248300]),[8] PPK Gamborg Nielsen (Norrbotten recessive type PPK [MIM 244850]),[9,10] and acral keratoderma.[11] These other

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