(patient [Pt] 8, 9, 21, 28, and 43), whose clinical features have been previously reported [14–17], were also included in this study. With the questionnaire survey for attending physicians, we accumulated the patients' clinical information, including craniofacial/skeletal features, neurodevelopmental features, brain structural abnormalities, cardiac abnormalities, sensory-organs abnormalities, urogenital abnormalities, endocrinological and nutritional findings among others. This study was approved by the ethics committee in Tokyo Women's Medical University.

On receipt of written informed consents from the families of the patients, we obtained the patients' blood samples to determine genomic copy number losses in the patients. Genomic DNA was extracted from the blood samples using the QIA quick DNA Extraction Kit (QIAGEN, Hamburg, Germany). Metaphase spreads were also prepared from blood samples and used for FISH analyses. In cases, if we could obtain written informed consent, parental samples were also analyzed.

2.2. Molecular and cytogenetic analyses

Chromosomal microarray testing was performed using any of the Agilent Oligo Microarray Kits 44, 60, 105, 180, and 244 K (Agilent Technologies, Santa Clara, CA), as described previously [18,19]. Genomic copy number aberrations were visualized using Agilent Genomic Workbench version 6.5 (Agilent Technologies). For cases in which variations of unknown significance were identified or suspected, parental samples were also analyzed. In cases of complex chromosomal rearrangements or mosaicism, metaphase spreads prepared using the patients' samples were used for FISH analyses for confirmation. The bacterial artificial clones were selected from the UCSC genome browser (http://genome.ucsc.edu/) for use as probes. For the target probes, RP11-425E15 (1p36.33: 949,400–1,132,489), RP11-82D16 (1p36.33: 2,046,751–2,208,312), RP11-70N12 (1p36.32: 2,740,703–2,922,551), CTD-3209F18 (1p36.32: 3,530,092–3,769,006), and RP11-933B18 (1p36.31: 5,988,719–6,177,261) were selected, while CTB-167K11 (1q44: 249,250,621–249,250,621) was used as a marker of chromosome 1. All of the genomic regions are described according to the February 2009 human reference sequence (GRCh37/hg19) in this study.

3. Results

3.1. Molecular-cytogenetic findings

We obtained clinical information from 86 patients with chromosomal deletions involving 1p36 regions. Among them, 50 patients (15 males and 35 females) were successfully genotyped. All of the genotypes were summarized in Tables 1 and 2, and 1p36 deletions identified in the patients were depicted in the genome map (Fig. 2). The minimum and maximum deletion sizes was 0.9 and 12.9 Mb, respectively. Pure terminal deletions were identified in 38 patients (76%). Among them, three patients (Pt 8, 19, and 21) exhibited mosaicism. Pt 8 was first diagnosed with mosaic 1p36 deletion by chromosomal microarray testing, and Pt 21 had been diagnosed with 1p36 deletion using subtelomere FISH analysis; however, mosaicism was not reported at that time [17]. Although the mosaic deletion of 1p36 in Pt 19 had been firstly confirmed by FISH, we could not detect the breakpoint by chromosomal microarray testing due to low frequency (28% mosaic ratio). As the breakpoint was determined to be between two FISH probes (CTD-3209F18 and RP11-933B18), the proximal end of CTD-3209F18 was used as the minimum deletion region in this patient.

Additional aberrations with the sizes over 0.5 Mb were identified in eight patients (Pt 2, 10, 11, 15, 20, 28, 34, and 43) involving chromosomes 4, 7, 8, 13, and Y (Table 2), including a possible benign copy number aberration in Pt 15, which was also observed in the healthy mother. The other seven patients were confirmed to have unbalanced translocations by cytogenetic evaluation (14%), using either G-banding or FISH analysis. Two translocations were diagnosed as de novo, and the others were designated as unknown because of the lack of availability of parental information.

Five patients (Pt 1, 14, 47, 48, and 50) had interstitial deletions (10%) with a deletion size between 0.9 and 10.3 Mb.

3.2. Clinical findings

Clinical information of the 50 patients successfully genotyped is summarized in Table 3. Estimated frequencies of each complication are also included in Table 3. Pt 26 and 49 suddenly died at 24 and 10 months old of age, respectively. Pt 49 probably died due to heart failure but Pt 26 died of an unknown cause (detailed information unavailable).

3.2.1. Craniofacial features

Most of the patients showed craniofacial features, including straight eyebrows (84%), deep-set eyes (93%), broad nasal bridge (97%), low set ears (88%), and a pointed chin (89%). Constellations of these findings make distinctive facial impressions for 1p36 deletion syndrome, observed in Pt 3, 6, and 14 (Fig. 1b–d). This observation suggests that hypotelorism is rather characteristic among these patients. On the other hand, Pt 1 did not show deep-set eyes (Fig. 1a). The craniofacial features of three patients (Pt 47, 48, and 50) did not exhibit hypotelorism (Fig. 2e–g). From the genotypic point of view, these three patients (Pt 47, 48, and 50) would be diagnosed as having the proximal 1p36 deletion syndrome [20,21].

Table 1
The ranges of 1p36 deletions analyzed by chromosomal microarray testing.

Patient number	Age (year)	Gender	Platform (k)	Start[a]	End[a]	Additional aberration	FISH probe	Mosaic ratio[b] (%)	References
1	14	F	180	834,101	1,770,669	Interstitial	RP11–425E15		
2	9	M	44	1	1,820,584	der(1)t(Y;1), idic(Y)			
3	6	F	180	1	2,186,829				
4	1	F	60	1	2,239,497				
5	3	F	44	1	2,281,699				
6	5	F	60	1	2,553,982				
7	2	M	60	1	2,553,982				
8	5	F	44	1	3,044,953	Mosaicism	RP11–82D16	70	Shimada et al. [17]
9	13	F	44	1	3,102,718				Okamoto et al. [14]
10	18	F	44	1	3,102,718	der(1)t(1;7)			
11	17	F	60	1	3,138,565	der(1)t(1;8)			
12	8	F	60	1	3,265,702				
13	11	F	244	1	3,408,152				
14	5	M	60	1,786,789	3,472,907	Interstitial			
15	2	F	180	1	3,564,328				
16	13	M	60	1	3,582,084				
17	4	F	44	1	3,607,275				
18	2	F	60	1	3,660,110				
19	3	F	60	1	3,769,006	Mosaicism	CTD-3209F18	28	
20	3	M	44	1	4,070,842	der(1)t(1;13)			
21	17	F	44	1	4,481,324	Mosaicism	RP11–82D16	77	Shimada et al. [17]
22	2	F	180	1	4,703,581				
23	6	M	60	1	4,779,157				
24	3	F	60	1	4,843,370				
25	6	F	44	1	4,843,718				
26	2	M	60	1	5,252,985				
27	0	F	44	1	5,252,985				
28	25	F	44	1	5,411,803	der(1)t(Y;1)			Hiraki et al. [15]
29	3	F	44	1	6,128,223				
30	3	F	60	1	6,282,562				
31	1	F	60	1	6,282,562				
32	3	M	60	1	6,882,431				
33	7	M	60	1	7,035,075				
34	1	F	60	1	7,187,535	der(1)t(1;4)			
35	10	M	60	1	7,392,688				
36	8	M	60	1	7,581,058				
37	3	F	44	1	8,077,959				
38	2	F	60	1	8,104,671				
39	3	M	44	1	8,104,671				
40	4	M	44	1	8,181,042				
41	5	F	44	1	8,181,042				
42	1	F	60	1	8,427,633				
43	3	M	60	1	9,180,975	der(1)(1;4)			Saito et al. [16]
44	5	F	60	1	9,251,936				
45	4	M	60	1	9,953,030				

46	2	F	44		1	10,001,011		
47	4	F	44		2,080,309	10,869,155	Interstitial	
48	8	F	60		2,785,042	12,743,178	Interstitial	
49	0	F	44		1	12,917,483		
50	22	F	180		6,614,950	16,890,814	Interstitial	

[a] The genomic position reffering build19.
[b] The mosaic ratio was confirmed by FISH; F, female; M, male.

3.2.2. Neurological features

Almost all patients showed ID (98%) but a patient (Pt 2) having a deletion in the far distal region of 1p36 showed borderline ID, with an intelligence quotient (IQ) of 80. Therefore, this region could be eliminated from the responsible region for ID (Fig. 2). The smallest deletion, an interstitial deletion between genomic positions 0.8 and 1.8 Mb, was identified in Pt 1 (Fig. 2). In spite of having this smallest deletion, Pt 1 had severe ID, i.e., she was locomotive but aphasic and required support for all activities in her daily life. This was probably a consequence of intractable epilepsy associated with tonic seizures, caused by factors other than the interstitial deletion of this region. The proximal and distal ends of the breakpoints in Pt 3 and 14 defined the shortest region of overlap for ID, spanning the 1.8–2.2 Mb region (Fig. 2; region B). Axial hypotonia (92%) and poor sucking (70%) were also commonly observed. Epilepsy, one of the major complications in 1p36 deletion syndrome, was observed in 70% of the patients. Infantile spasms were observed in 16% of the patients.

In this study, many types of structural brain abnormalities were identified; not only in the cerebral cortex but also in the white matter (Table 3), indicating that there is no major pattern. The most frequently observed abnormality was a nonspecific finding with enlargement of lateral ventricles.

3.2.3. Cardiac abnormality

Cardiac abnormality is one of the most frequently observed complications in patients with 1p36 deletions. In this study, congenital heart defects and functional abnormalities were observed in 69% (34/49) and 22% (11/49) of the patients, respectively. The most frequently observed patterns were patent ductus arteriosus (PDA; 37% [18/49]) and ventricular septal defects (VSD; 37% [18/49]).

3.2.4. Other complications

Many kinds of complications were observed in many organs. Cryptorchidism was the most frequently observed complication in male patients (64% [9/14]). As Pt 14, with a small interstitial deletion spanning from 1.8 to 3.5 Mb, had cryptorchidism, the deleted region was likely involved in abnormalities of the external genitalia (Fig. 2; region H). Hearing problems (39% [19/49]) and strabismus (33% [15/46]) were relatively common among the patients. Obesity was observed in 5 patients (11% [5/46]).

Renal abnormalities were rare and identified only in three patients. Among them, Pt 26, who had a 5.3 Mb deletion, was diagnosed with the autosomal recessive cystic kidney disease of nephronophthisis (this patient died at 2 years of age) [22]. One of the genes responsible for this condition, the nephronophthisis 4 gene

Table 2
Additional aberrations identified in the patients.

Patient number	Chr	Start[a]	End[a]	Remark	Attribute	Origin
2	Y	1	59,373,566	der(1)t(Y;1)(p36.3;q12), idic(Y)(q12)	dup	NA
10	7	1	6,870,943	der(1)t(1;7)(p36.32;p22.1)	dup	NA
11	8	1	3,909,039	der(1)t(1;8)(p36.22;p23.2)	dup	NA
15	1	146,324,068	149,192,104	del(1)(q21.1;q21.2)	del	Common with mother
20	13	100,462,233	115,169,878	der(1)t(1;13)(p26.32;q32.3)	dup	De novo
28	Y	26,435,039	59,373,566	der(1)t(Y;1)(q12;p36.32)[#]	dup	NA
34	4	1	13,396,747	der(1)t(1;4)(p36.31;p15.33)	dup	De novo
43	4	189,012,426	191,154,276	der(1)t(1;4)(p36.31;q35.2)	dup	NA

[*] The genomic position reffering build19; dup, duplication; del, deletion; NA, not available.
[#] This case was previously reported by Hiraki et al. [15].

(*NPHP4*), is located on 1p36 (chr1: 5,946,555–5,965,543) [23], proximal to the deletion region of three patients with renal abnormalities (Pt 26, 33, and 35). It is unclear whether there is a correlation between *NPHP4* and the renal abnormalities observed in this study.

4. Discussion

4.1. Previous genetic studies on the 1p36 deletion syndrome

Many cohort studies have been performed to delineate the phenotypic features of patients with 1p36 deletion syndrome and to evaluate the frequency of complications [1,6,7]. It has been reported that there is no correlation between the deletion size and the number of observed clinical features [24], while the critical region responsible for core phenotypic features, including clefting, hypothyroidism, cardiomyopathy, hearing loss, large fontanel, and hypotonia, has been narrowed down to a region 2.2 Mb from the telomere [3]. Compared to such core phenotypic features, other complications tend to vary with the size of the deletion, and study subjects with larger deletions tend to have more phenotypic features [25], suggesting that the various phenotypic features are dependent on genes involved in the deletion regions. Thus, precise knowledge of the genotype–phenotype correlations could potentially lead to more personalized treatments for individuals with 1p36 deletions and might identify mutations for single gene disorders [3]. The potassium voltage-gated channel, shaker-related subfamily, beta member 2 gene (*KCNAB2*) and the v-ski sarcoma viral oncogene homolog gene (*SKI*) were identified as candidate genes for the epilepsy phenotype and clefting abnormalities, respectively [26,27]. More recently, the PR domain containing 16 gene (*PRDM16*) was identified as a possible candidate gene for cardiomyopathy, as *PRDM16* was included in a minimal deletion among patients with 1p36 deletions associated with cardiomyopathy, while in patients with pure cardiomyopathy, single nucleotide variants of *PRDM16* were identified as the cause of cardiomyopathy [28]. This was one of the most successful studies of genotype–phenotype correlation in patients with 1p36 deletions [28].

4.2. Craniofacial features

As mentioned above, a region 2.2 Mb from the telomere has been reported to be responsible for core phenotypic features of 1p36 deletion syndrome [3]. Compared to this, we observed atypical facial features in four patients (Pt 1, 47, 48, and 50) whose deletions did not include the 1.8–2.1 Mb region, in this study. Thus, the region responsible for typical facial features is narrowed into this region (Fig. 2; region A). Because hypotelorism has never been listed in the clinical delineations of 1p36 deletion syndrome reported from Western countries, we did not include this finding in the questionnaire survey and the frequency of this finding in Japanese patients could not be calculated. However, it is commonly observed in Japanese patients with typical 1p36 deletion syndrome. Therefore, hypotelorism may be a characteristic finding among Asian patients.

4.3. Neurological features

Although more severe ID was reported to be associated with larger 1p36 deletions [10], the genomic region responsible for severe ID has never been identified. In this study, a patient (Pt 28) having a 5.4 Mb deletion acquired independent gait, while patients with >6.1 Mb deletions had not yet acquired independent gait, and exhibited severe ID. Thus, the region between 5.4 and 6.1 Mb would appear to be the borderline for independent gait (Fig. 2; region C), and the modifier genes for prognosis of development may be located in the region proximal to this borderline. *KCNAB2*, mentioned above, may be one of the modifier genes responsible for severe ID. Chromodomain helicase DNA-binding protein 5 (*CHD5*; chr1: 6,161,847–6,240,194), which encodes a neuron-specific protein, is

Table 3
Summary of clinical features of the patients with 1p36 deletions.

Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	Frequencies
1. Craniofacial and skeletal features																																																		
Characteristic craniofacial features																																																		
Microcephaly	NA	+	NA	+	+	NA	NA	–	+	NA	+	NA	NA	+	NA	NA	+	+	NA	NA	NA	NA	NA	NA	NA	NA	+	+	+	NA	–	–	+	NA	NA	NA	+	NA	+	NA	+	NA	–	NA	+	NA	+	+	+	+	83% (20/24)
Brachycephaly	+	NA	–	+	+	NA	NA	–	+	NA	+	NA	NA	–	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	+	NA	NA	NA	NA	NA	NA	–	NA	NA	+	NA	NA	NA	NA	+	+	+	65% (11/17)
Straight eyebrow	–	+	+	+	+	+	+	+	+	+	+	–	+	+	NA	+	+	+	NA	+	+	+	+	+	+	NA	NA	+	+	NA	+	+	+	+	NA	NA	NA	NA	+	+	+	NA	+	+	NA	NA	–	–	+	–	84% (32/38)
Deep-set eyes	–	+	+	+	+	+	+	+	+	+	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+	+	NA	NA	+	+	+	+	+	+	+	+	NA	+	+	+	NA	NA	+	NA	+	NA	+	NA	+	–	+	–	93% (37/40)
Epicanthus	+	NA	+	+	+	+	+	+	+	NA	+	+	+	–	NA	NA	+	+	NA	+	–	+	+	+	NA	NA	NA	NA	+	+	+	–	–	NA	+	NA	NA	NA	+	NA	+	+	NA	+	+	NA	–	+	+	+	86% (30/35)
Broad nasal root/bridge	+	NA	+	+	NA	+	+	+	+	NA	+	+	+	–	NA	NA	+	+	+	+	+	NA	+	NA	NA	NA	NA	+	+	+	+	+	+	NA	NA	+	NA	NA	+	+	NA	+	NA	+	NA	+	+	+	+	97% (32/33)
Long philtrum	+	+	+	–	NA	–	–	+	+	+	+	–	–	–	NA	NA	+	+	+	+	+	+	+	NA	NA	NA	NA	NA	+	–	NA	–	–	+	+	NA	+	NA	NA	–	NA	NA	+	NA	+	+	–	–	+	–	63% (22/35)
Low set ears	–	–	+	–	NA	–	+	NA	+	NA	+	+	NA	+	NA	NA	NA	NA	NA	+	+	+	+	+	NA	+	+	+	+	+	+	+	+	+	+	+	+	NA	NA	+	NA	NA	NA	NA	+	+	+	+	+	88% (29/33)
Pointed chin	+	+	+	+	NA	+	+	+	+	+	+	+	+	+	NA	NA	+	+	NA	+	+	+	+	NA	+	NA	+	+	+	+	+	–	+	+	+	NA	NA	NA	NA	+	+	+	–	NA	+	+	–	–	+	–	89% (34/38)
Late closure of the anterior fontanel	–	NA	+	–	–	–	NA	NA	+	NA	NA	NA	NA	NA	+	NA	+	NA	+	+	+	–	–	NA	NA	–	+	+	–	+	+	+	+	+	–	+	NA	NA	–	+	–	–	–	+	NA	+	NA	–	NA	NA	56% (18/32)
Cleft palate problem																																																		
High palate	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	+	–	+	–	+	+	–	+	–	+	–	+	–	+	+	–	–	+	+	29% (14/49)
Cleft palate	–	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–	+	–	–	–	+	–	–	–	–	–	–	+	–	NA	–	–	–	–	+	–	+	–	–	+	–	+	–	+	–	+	–	–	–	–	22% (11/49)
Cleft lip	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	NA	–	–	–	–	+	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	16% 8/(49)
Cleft jaw	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	2% (1/50)
Limb abnormalities																																																	
Finger abnormalities	–	–	NA	–	–	–	–	–	–	–	–	NA	–	+	–	+	–	+	–	–	–	–	+	–	–	+	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15% (7/48)
Limb deformity	–	–	NA	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	4% (2/48)
Other skeletal abnormalities																																																	
Rib abnormalities such as 11 ribs	–	–	NA	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	4% (2/48)
Scoliosis	–	–	NA	–	–	–	–	–	+	–	–	NA	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–	+	+	–	+	25% (12/48)
Congenital dislocated hip	–	–	NA	–	–	–	–	–	+	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4% (2/48)
Developmental dysplasia of the hip	–	–	NA	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	2% (1/48)
2. Neurological features (clinical)																																																	
Axial hypotonia	+	+	NA	–	+	+	+	+	+	+	+	+	+	NA	+	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	92% (44/48)
Poor sucking	–	–	NA	–	–	–	+	–	+	+	+	NA	+	NA	+	NA	+	+	–	+	+	–	–	–	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	70% (32/46)
Difficulty of swallowing	–	–	+	–	–	–	+	–	+	–	+	NA	–	NA	+	+	+	+	–	+	+	–	–	–	–	+	–	+	–	+	–	+	+	+	+	+	+	–	+	+	–	+	–	+	+	+	+	+	+	56% (27/48)
Developmental delay																																																	
Intellectual disability	S	B	S	S	M	S	S	M	S	S	S	S	S	M	S	S	M	S	S	S	M	S	M	M	S	S	NA	S	M	S	S	S	S	S	S	S	S	NA	S	M	S	S	S	S	S	S	NA	S	S	98% (49/50)
Acquire independent gait	+	+	+	–	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	32% (16/50)
Expressive language																																																	
Sentence	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6% (3/50)
Only words	–	–	–	–	+	+	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	16% (8/50)
Dysarthria	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8% (4/50)
Gestures	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10% (5/50)
Behavior disorders																																																	
Self–injury	–	+	+	–	–	NA	+	–	NA	–	+	NA	+	NA	–	NA	–	NA	+	–	–	NA	–	NA	–	–	NA	+	+	+	–	–	NA	–	–	–	NA	–	–	–	NA	–	–	–	NA	–	–	–	30% (11/37)
Temper tantrum	+	–	–	–	–	NA	+	+	NA	+	+	NA	+	NA	–	NA	–	NA	–	–	+	NA	+	NA	–	–	NA	+	–	–	–	–	NA	–	–	–	NA	–	–	–	NA	–	–	–	NA	–	–	–	30% (11/37)
Poor social interaction	+	–	+	+	–	NA	–	+	NA	–	+	NA	+	NA	–	NA	–	NA	–	+	–	NA	–	NA	–	–	NA	+	–	–	–	+	NA	–	–	–	NA	–	–	–	–	–	–	–	NA	+	–	–	NA	–	27% (10/37)
Epilepsy																																																	
History of epilepsy	+	–	+	+	–	–	+	–	–	+	+	+	–	+	+	+	–	+	–	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	–	+	–	+	–	+	+	+	+	–	+	+	70% (35/50)
Infantile spasms	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	+	–	–	–	+	–	+	–	–	–	–	–	16% (8/50)
3. Neurological features (radiological)																																																	
Cerebral cortex																																																	
Periventricular nodular heterotopia (PVNH)	–	–	NA	–	–	–	–	+	–	NA	–	–	+	–	NA	NA	+	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	–	–	+	–	–	–	–	–	–	–	–	–	–	NA	–	10% (4/42)
Polymicrogyria	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	–	–	–	–	–	–	+	NA	–	–	NA	–	–	NA	–	–	–	–	+	–	–	–	–	–	–	–	–	–	NA	–	7% (3/42)
Cortical dysplasia	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	+	NA	–	–	–	–	–	–	–	+	–	–	–	–	–	NA	–	5% (2/42)
Cortical hypoplasia	–	–	NA	–	–	–	–	–	–	NA	–	+	–	–	NA	NA	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	–	–	–	–	–	+	–	+	–	–	–	–	–	NA	–	7% (3/42)
Cerebral white matter																																																	
Enlargement of lateral ventricles	–	–	NA	+	–	–	–	–	–	NA	–	–	+	+	NA	NA	–	–	–	+	–	–	–	–	–	–	NA	–	+	NA	–	+	NA	+	+	–	+	–	+	–	–	+	+	+	+	NA	–	36% (15/42)
Delay in myelination	–	–	NA	–	–	–	–	–	–	NA	–	–	–	+	NA	NA	+	–	–	–	–	–	–	–	+	NA	–	+	–	NA	+	+	NA	–	+	–	+	–	–	–	+	–	–	+	NA	–	21% (9/42)
Hypoplasia of corpus callosum	–	–	NA	+	–	–	–	–	–	NA	–	–	–	+	NA	NA	–	–	–	+	–	–	–	–	+	NA	–	+	NA	–	–	NA	–	–	–	+	–	+	–	+	–	–	–	–	+	+	NA	–	24% (10/42)
Cerebellum																																																	
Chiari type II malformation	–	–	NA		–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	2% (1/42)
Others																																																	
Cavum septum pellucidum	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	+	–	–	–	–	–	–	+	–	–	NA	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	5% (2/42)
Choroid plexus cyst	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	+	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	5% (2/42)
Arrested hydrocephalus	–	–	NA	–	–	–	–	–	–	NA	–	–	–	+	NA	NA	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	2% (1/42)
Enlargement of subdural space	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	–	–	–	–	–	–	+	–	NA	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	2% (1/42)
Arachnoid cyst	–	–	NA	–	–	–	–	–	–	NA	–	–	–	–	NA	NA	–	–	–	–	–	–	–	–	–	+	NA	–	–	NA	–	–	NA	–	–	+	–	–	–	–	–	–	–	–	–	–	NA	–	5% (2/42)
4. Cardiac abnormalities																																																	
Congenital heart defects																																																	
Patent ductus arteriosus (PDA)	–	–	–	–	–	–	–	–	–	+	+	+	–	–	NA	–	–	–	+	–	–	+	+	–	–	+	–	+	+	–	+	–	+	–	+	+	–	+	–	–	+	–	–	+	–	+	–	–	+	37% (18/49)
Ventricular septal defects (VSD)	–	–	–	–	–	+	–	+	–	–	–	+	–	–	NA	+	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	+	+	+	+	–	–	+	+	–	–	+	–	–	+	+	+	+	–	–	37% (18/49)
Artial septal defects (ASD)	–	–	–	–	–	–	–	+	–	–	–	–	–	–	NA	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	+	+	–	–	+	–	+	–	–	–	–	–	–	+	–	–	–	16% (8/49)
Aortic stenosis (AS)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	4% (2/49)
Pulmonary stenosis (PS)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	4% (2/49)
Aortic valve prolapse	–	–	–	–	–	–	–	–	–	–	–	+	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)
Ebstein anomaly	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	6% (3/49)
Double outlet right ventricle (DORV)	–	–	–	–	–	+	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	6% (3/49)
Hypoplasia of the left ventricle (HLHS)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)
Patent foramen ovale (PFO)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)
Partial anomalous pulmonary venous connection (PAPVC)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)

Pulmonary atresia (PA)	–	–	–	–	–	+	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/49)
Positional anomaly of valsalva sinus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	2% (1/49)
Functional abnormalities																																																			
Pulmonary hypertension (PH)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	8% (4/49)
Left ventricular noncompaction (LVNC)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	10% (5/49)
Dilated cardiomyopathy	–	–	–	–	–	–	–	–	+	–	+	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4% (2/49)
5. Sensory organs																																																			
Ears																																																			
Hearing problems	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	+	–	–	+	–	–	–	+	+	–	–	+	–	–	NA	+	–	+	+	+	–	–	+	+	+	+	+	–	–	–	+	–	39% (19/49)
Eyes																																																			
Strabismus	–	–	+	–	–	–	+	NA	–	–	+	–	+	–	–	–	+	+	–	–	+	–	–	+	–	–	–	–	+	NA	–	–	NA	+	–	–	+	–	–	+	+	–	NA	–	–	+	–	–	–	+	33% (15/46)
Ametropia	–	–	+	–	–	–	–	NA	–	–	+	–	–	+	–	–	+	+	+	–	+	–	+	–	+	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	+	–	–	NA	–	–	+	–	+	–	–	26% (12/46)
Nystagmus	–	–	–	–	–	–	–	NA	+	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	NA	–	–	NA	+	–	–	–	–	–	+	–	–	NA	+	+	–	+	–	–	–	20% (9/46)
Oculomotor disturbance	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	+	–	–	–	–	2% (1/46)
Coloboma	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	+	–	–	–	–	–	NA	–	–	–	–	–	–	–	2% (1/46)
Congenital microphthalmia	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	+	–	2% (1/46)
Retinitis pigmentosa	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	NA	–	–	NA	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	2% (1/46)
6. Urogenital abnormalities																																																			
Renal abnormalities																																																			
Renal hypoplasia	–	–	NA	–	–	–	NA	NA	–	+	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Hydronephrosis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	NA	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5% (2/43)
Pyelectasis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	NA	+	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Nephronophthisis	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	NA	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/43)
Extragenital abnormalities																																																			
Ectopic kidney	–	–	NA		–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2% (1/44)
Ambiguous external genitalia	–	–	NA	–	–	–	NA	NA	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	NA	+	–	–	–	–	–	–	–	–	+	+	–	–	NA	–	–	7% (3/43)
Cryptorchidism	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	–	NA	NA	NA	+	NA	NA	+	NA	NA	+	NA	NA	NA	NA	NA	+	–	NA	–	+	NA	NA	+	+	NA	NA	–	NA	+	NA	NA	NA	NA	NA	64% (9/14)
Scrotal hypoplasia	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	NA	–	NA	NA	NA	–	NA	NA	–	NA	NA	–	NA	NA	NA	NA	NA	–	–	NA	+	–	NA	NA	–	–	NA	NA	–	NA	+	NA	NA	NA	NA	NA	14% (2/14)
7. Endocrinology and nutrition																																																			
Hypothyroidism	–	–	NA	–	–	–	NA	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	NA	+	–	–	+	+	NA	–	–	–	+	–	–	–	NA	–	–	16% (7/45)
Obesity	–	–	NA	–	NA	–	–	+	–	+	+	–	+	–	–	–	–	–	–	NA	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NA	–	–	–	–	–	–	–	–	–	–	11% (5/46)
8. Other complications																																																			
Respiratory tract related																																																			
Stenosis of nasal cavity	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	
Laryngomalacia and/or tracheomalacia	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Adenoidal hypertrophy and/or enlarged tonsil	NA	+	NA	–	NA	NA	NA	+	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Obstructive sleep apnea	NA	NA	NA	–	NA	NA	NA	+	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Allergy related																																																			
Atopic dermatitis	NA	NA	NA	–	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	+	NA	NA	–	–	NA	+	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Food allergy	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Bronchial asthma	NA	+	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Related to secondary																																																			
Precocious puberty	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Breast enlargement	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Others																																																			
Atresia of external acoustic foramen	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	
Dental abnormalities	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	
Non alcoholic steatohepatitis (NASH)	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Constipation	NA	NA	NA	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	–	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	NA	

NA, not available; S, severe; B, borderline; M, moderate.

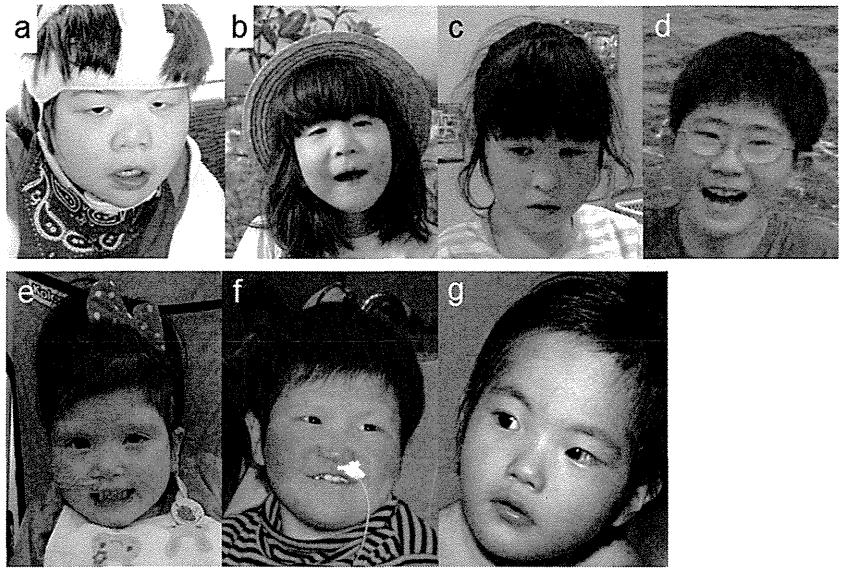

Fig. 1. Facial features of the patients with variably sized 1p36 deletions. Pt 1 (a; at 14 years of age) shows edematous eyelids rather than deep-set eyes. Pt 3 (b; 6 years), 6 (c; 5 years), and 14 (d; 15 years) share characteristic features, including deep-set eyes, hypotelorism, and pointed chins. Pt 47 (e; 4 years) and 48 (f; 8 years) do not exhibit such characteristic features, with round faces rather than hypotelorism and pointed chins. Pt 50 (g; 3 years) exhibits distinctive features with arched eyebrows and hypertelorism. Written informed consent to publish patient photos was obtained from all the patient families.

involved in chromatin remodeling and gene transcription, regulating the expression of neuronal genes [29]. Thus, *CHD5* also may be a modifier gene for severe ID.

It has been suggested that two genes, gamma-aminobutyric acid (GABA) A receptor delta (*GABRD*; chr1: 1,950,768–1,962,192), and *KCNAB2* (chr1: 6,105,981–6,161,253), are associated with the manifestations of epilepsy [27]. This is also been suggested by our present study, as there was no history of epilepsy in a patient (Pt 2) with a 1.8 Mb terminal deletion and a patient (Pt 50) with a 10.0 Mb interstitial deletion; both of the deletions includes neither *GABRD* nor *KCNAB2* (Fig. 2). The incidence of epilepsy was higher in the patients with severe ID (30/38; 79%) than in the patients with moderate ID (4/8; 50%). Thus, the severity of ID was associated with the incidence of epilepsy and the same gene/set of genes may be involved in both of these neurological manifestations.

Several case reports have suggested an association between periventricular nodular heterotopia (PVNH) and 1p36 deletion [16,30–32], and the candidate region for polymicrogyria has been mapped to the distal 4.8 Mb region [33]. As the smallest deletion among the patients with abnormal neuronal migration was 3.0 Mb (Pt 8), the gene(s) responsible for this phenotype may be narrowed down to the distal 3.0 Mb region (Fig. 2; region D). Chiari malformation type II was identified only in Pt 34, who showed an unbalanced translocation with chromosome 4. Thus, this rare feature may be attributable to the partial trisomy of chromosome 4.

4.4. Cardiac abnormality

Previously, the genetic region responsible for left ventricular noncompaction (LVNC) was assigned to the 1.9–3.4 Mb region [34–36]. On the other hand, there are many reports which show an association between Ebstein anomaly and 1p36 deletion [7,37–40]. The genomic region responsible for Ebstein anomaly was assigned to the 2.9–3.8 Mb region [39,40]. In 2005, Sinkovec et al. reported two patients with LVNC associated with Ebstein anomaly [41]. In this study, we identified a patient (Pt 24) who showed both LVNC and Ebstein anomalies. Given this perspective, it might be reasonable to conclude that the critical regions involved in LVNC and Ebstein anomaly are relatively close. As mentioned above *PRDM16* located on chr1: 2,985,742–3,355,185 was reported as a gene responsible for cardiomyopathy and LVNC [28]. This is in agreement with our study, as the smallest deletion identified in a patient (Pt 9) with DCM was 3.1 Mb in size. It is possible that *PRDM16* may also be related not only to LVNC but also to the Ebstein anomaly.

Although double-outlet right ventricle (DORV) has never been reported in individuals with 1p36 deletions, we found DORV in two patients. We found a relatively small deletion (2.5 Mb) in a patient (Pt 6) with DORV (Fig. 2; region D). There is a possibility that the protein kinase C zeta gene (*PRKCZ*; chr1 chr1: 1,981,909–2,116,834) is related to cardiac abnormalities, because this gene had been implicated in a variety of process including cardiac muscle function [42,43]. The positional

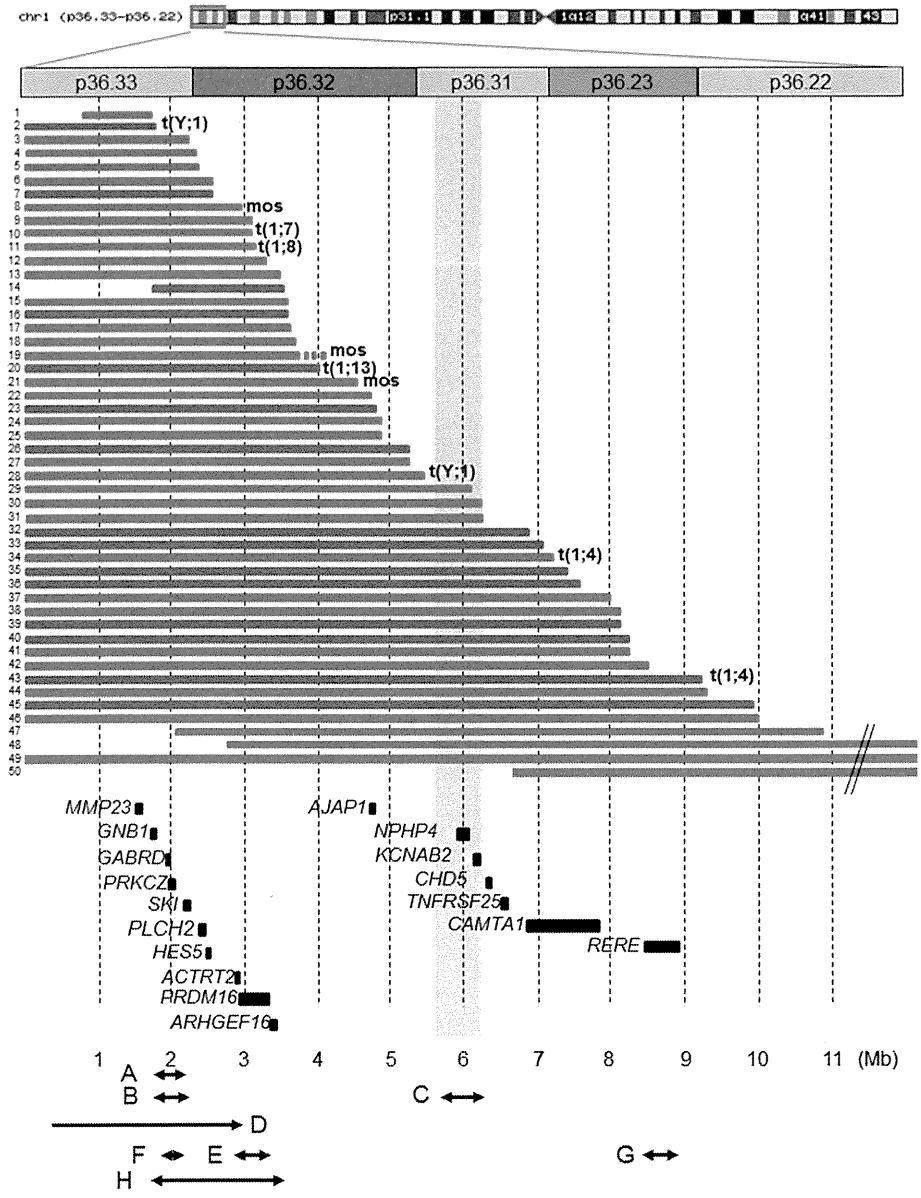

Fig. 2. Result of chromosomal microarray testing depicted in a genome map of the 1p36 region. The scheme of chromosome 1 (top) is downloaded from the UCSC genome browser. Red and blue bars indicate the deletion regions identified in female and male patients, respectively. Black bars indicate the locations of the genes, discussed in the text. The numbers depicted on the left side of each bar indicate patients' numbering. "t" and "mos" indicate unbalanced translocations and mosaicism, respectively. Yellow and green translucent vertical lines emphasize the proposed responsible regions for ID. Proposed responsible regions for each phenotype; A, distinctive craniofacial findings; B, ID; C, modifier effect for ID; D, LVNC and Ebstein anomaly; E, DORV; F, cardiac anomalies; G, cryptochidisms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effects for *PRDM16* may be another possibility in this case.

The arginine-glutamic acid dipeptide (RE) repeats gene (*RERE*; chr1: 8,412,464–8,877,699) has been reported to play a critical role in early cardiovascular development [44]. In this study, all patients with deletions larger than 8.4 Mb, which involve *RERE*, showed cardiac anomalies. Thus, *RERE* may be involved in the pathogenesis of congenital heart defects (Fig. 2; region G).

Only Pt 20, with an unbalanced translocation between 13q32.3, showed hypoplasia of the left ventricle (HLHS) in this study. HLHS accounts for 2–3% of all congenital heart defects, and a minority of HLHS cases have been associated with congenital anomaly syndromes, e.g., the Jacobsen, Turner, and Potocki–Lupski syndromes, respectively [45–47]. As 13q duplication has been reported to be associated with this manifestation, the findings of HLHS found in Pt 20 may be due to a partial trisomy of 13q [48].

4.5. Other complications

In patients with 1p36 monosomy, a Prader–Willi syndrome (PWS)-like phenotype has been described [6,13,49]. The clinical features that overlap between the 1p36 deletion syndrome and PWS are ID, neonatal hypotonia, obesity, craniofacial anomalies, hyperphagia, short stature, and behavior problems. D'Angelo et al. described a patient with a 2.5 Mb deletion within the chromosome region 1p36.33–1p36.32 [13]. Tsuyusaki et al. hypothesized that the critical region for the PWS-like phenotype was within 4 Mb from 1pter [49]. Rosenfeld et al. suggested a critical region for the PWS-like phenotype in the 1.7–2.3 Mb region [12]. In this study, all five patients with obesity (Pt 8, 10, 11, 13, and 21) were female, and acquired ambulatory ability within the ages of 2–8 years. Two of the patients (Pt 8 and 21) showed mosaic deletions [17]. From these perspectives, we speculate that female patients who showed 1p36 deletions involving the critical region and who acquired ambulatory ability are likely to be at risk for obesity.

5. Conclusion

In this study, we successfully accumulated the genotype–phenotype data of 50 patients with the deletions of 1p36 regions. As hypotelorism was commonly observed in patients, it may be characteristic of Asian patients. The genotype–phenotype correlation analysis narrowed down the regions responsible for distinctive craniofacial features and ID to the 1.8–2.1 and 1.8–2.2 Mb regions, respectively. Patients with deletions larger than 6.2 Mb showed no ambulation, indicating that severe neurodevelopmental prognosis may be modified by haploinsufficiencies of *KCNAB2* and/or *CHD5*, located 6.2 Mb away from the telomere. Although the genotype–phenotype correlation for the cardiac abnormalities is unclear, *PRDM16*, *PRKCZ*, and *RERE* may be related to this complication. One more finding revealed by this study for the first time, is that female patients who acquired ambulatory ability are likely to be at a risk for obesity.

Acknowledgments

We would like to express our gratitude to the patients and their families for their cooperation. We also thank the Japanese Society of Child Neurology and the Japan Society of Pediatric Genetics for their supports. This work was supported by a Grant-in-Aid for Scientific Research from Health Labor Sciences Research Grants from the Ministry of Health, Labor, and Welfare, Japan.

References

[1] Shapira SK, McCaskill C, Northrup H, Spikes AS, Elder FFB, Sutton VR, et al. Chromosome 1p36 deletions: the clinical phenotype and molecular characterization of a common newly delineated syndrome. Am J Hum Genet 1997;61:642–50.
[2] Gajecka M, Yu W, Ballif BC, Glotzbach CD, Bailey KA, Shaw CA, et al. Delineation of mechanisms and regions of dosage imbalance in complex rearrangements of 1p36 leads to a putative gene for regulation of cranial suture closure. Eur J Hum Genet 2005;13:139–49.
[3] Heilstedt HA, Ballif BC, Howard LA, Lewis RA, Stal S, Kashork CD, et al. Physical map of 1p36, placement of breakpoints in monosomy 1p36, and clinical characterization of the syndrome. Am J Hum Genet 2003;72:1200–12.
[4] Shao L, Shaw CA, Lu XY, Sahoo T, Bacino CA, Lalani SR, et al. Identification of chromosome abnormalities in subtelomeric regions by microarray analysis: a study of 5,380 cases. Am J Med Genet A 2008;146A:2242–51.
[5] Heilstedt HA, Ballif BC, Howard LA, Kashork CD, Shaffer LG. Population data suggest that deletions of 1p36 are a relatively common chromosome abnormality. Clin Genet 2003;64:310–6.
[6] Slavotinek A, Shaffer LG, Shapira SK. Monosomy 1p36. J Med Genet 1999;36:657–63.
[7] Battaglia A, Hoyme HE, Dallapiccola B, Zackai E, Hudgins L, McDonald-McGinn D, et al. Further delineation of deletion 1p36 syndrome in 60 patients: a recognizable phenotype and common cause of developmental delay and mental retardation. Pediatrics 2008;121:404–10.
[8] Battaglia A. Del 1p36 syndrome: a newly emerging clinical entity. Brain Dev 2005;27:358–61.
[9] Zenker M, Rittinger O, Grosse KP, Speicher MR, Kraus J, Rauch A, et al. Monosomy 1p36-a recently delineated, clinically recognizable syndrome. Clin Dysmorphol 2002;11:43–8.
[10] Kurosawa K, Kawame H, Okamoto N, Ochiai Y, Akatsuka A, Kobayashi M, et al. Epilepsy and neurological findings in 11 individuals with 1p36 deletion syndrome. Brain Dev 2005;27:378–82.
[11] Buck A, du Souich C, Boerkoel CF. Minimal genotype–phenotype correlation for small deletions within distal 1p36. Am J Med Genet A 2011;155A:3164–9.
[12] Rosenfeld JA, Crolla JA, Tomkins S, Bader P, Morrow B, Gorski J, et al. Refinement of causative genes in monosomy 1p36 through clinical and molecular cytogenetic characterization of small interstitial deletions. Am J Med Genet A 2010;152A:1951–9.
[13] D'Angelo CS, Da Paz JA, Kim CA, Bertola DR, Castro CIE, Varela MC, et al. Prader–Willi-like phenotype: investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. Eur J Med Genet 2006;49:451–60.
[14] Okamoto N, Toribe Y, Nakajima T, Okinaga T, Kurosawa K, Nonaka I, et al. A girl with 1p36 deletion syndrome and congenital fiber type disproportion myopathy. J Hum Genet 2002;47:556–9.
[15] Hiraki Y, Fujita H, Yamamori S, Ohashi H, Eguchi M, Harada N, et al. Mild craniosynostosis with 1p36.3 trisomy and 1p36.3 deletion syndrome caused by familial translocation t(Y;1). Am J Med Genet A 2006;140A:1773–7.
[16] Saito Y, Kubota M, Kurosawa K, Ichihashi I, Kaneko Y, Hattori A, et al. Polymicrogyria and infantile spasms in a patient with 1p36 deletion syndrome. Brain Dev 2011;33:437–41.
[17] Shimada S, Maegaki Y, Osawa M, Yamamoto T. Mild developmental delay and obesity in two patients with mosaic 1p36 deletion syndrome. Am J Med Genet A 2014;164A:415–20.
[18] Shimada S, Okamoto N, Hirasawa K, Yoshii K, Tani Y, Sugawara M, et al. Clinical manifestations of Xq28 functional

disomy involving MECP2 in one female and two male patients. Am J Med Genet A 2013;161A:1779–85.
[19] Shimada S, Okamoto N, Ito M, Arai Y, Momosaki K, Togawa M, et al. MECP2 duplication syndrome in both genders. Brain Dev 2013;35:411–9.
[20] Kang SH, Scheffer A, Ou Z, Li J, Scaglia F, Belmont J, et al. Identification of proximal 1p36 deletions using array-CGH: a possible new syndrome. Clin Genet 2007;72:329–38.
[21] Shimojima K, Paez MT, Kurosawa K, Yamamoto T. Proximal interstitial 1p36 deletion syndrome: the most proximal 3.5-Mb microdeletion identified on a dysmorphic and mentally retarded patient with inv(3)(p14.1q26.2). Brain Dev 2009;31:629–33.
[22] Benzing T, Schermer B. Clinical spectrum and pathogenesis of nephronophthisis. Curr Opin Nephrol Hypertens 2012;21:272–8.
[23] Otto EA, Ramaswami G, Janssen S, Chaki M, Allen SJ, Zhou W, et al. Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. J Med Genet 2011;48:105–16.
[24] Gajecka M, Mackay KL, Shaffer LG. Monosomy 1p36 deletion syndrome. Am J Med Genet C 2007;145C:346–56.
[25] Wu YQ, Heilstedt HA, Bedell JA, May KM, Starkey DE, McPherson JD, et al. Molecular refinement of the 1p36 deletion syndrome reveals size diversity and a preponderance of maternally derived deletions. Hum Mol Genet 1999;8:313–21.
[26] Colmenares C, Heilstedt HA, Shaffer LG, Schwartz S, Berk M, Murray JC, et al. Loss of the SKI proto-oncogene in individuals affected with 1p36 deletion syndrome is predicted by strain-dependent defects in $Ski^{-/-}$ mice. Nat Genet 2002;30:106–9.
[27] Heilstedt HA, Burgess DL, Anderson AE, Chedrawi A, Tharp B, Lee O, et al. Loss of the potassium channel beta-subunit gene, *KCNAB2*, is associated with epilepsy in patients with 1p36 deletion syndrome. Epilepsia 2001;42:1103–11.
[28] Arndt AK, Schafer S, Drenckhahn JD, Sabeh MK, Plovie ER, Caliebe A, et al. Fine mapping of the 1p36 deletion syndrome identifies mutation of *PRDM16* as a cause of cardiomyopathy. Am J Hum Genet 2013;93:67–77.
[29] Potts RC, Zhang P, Wurster AL, Precht P, Mughal MR, Wood III WH, et al. CHD5, a brain-specific paralog of Mi2 chromatin remodeling enzymes, regulates expression of neuronal genes. PLoS One 2011;6:e24515.
[30] Neal J, Apse K, Sahin M, Walsh CA, Sheen VL. Deletion of chromosome 1p36 is associated with periventricular nodular heterotopia. Am J Med Genet A 2006;140:1692–5.
[31] Saito S, Kawamura R, Kosho T, Shimizu T, Aoyama K, Koike K, et al. Bilateral perisylvian polymicrogyria, periventricular nodular heterotopia, and left ventricular noncompaction in a girl with 10.5–11.1 Mb terminal deletion of 1p36. Am J Med Genet A 2008;146A:2891–7.
[32] Descartes M, Mikhail FM, Franklin JC, McGrath TM, Bebin M. Monosomy1p36.3 and trisomy 19p13.3 in a child with periventricular nodular heterotopia. Pediatr Neurol 2011;45:274–8.
[33] Dobyns WB, Mirzaa G, Christian SL, Petras K, Roseberry J, Clark GD, et al. Consistent chromosome abnormalities identify novel polymicrogyria loci in 1p36.3, 2p16.1-p23.1, 4q21.21-q22.1, 6q26-q27, and 21q2. Am J Med Genet A 2008;146A:1637–54.
[34] Thienpont B, Mertens L, Buyse G, Vermeesch JR, Devriendt K. Left-ventricular non-compaction in a patient with monosomy 1p36. Eur J Med Genet 2007;50:233–6.
[35] Cremer K, Ludecke HJ, Ruhr F, Wieczorek D. Left-ventricular non-compaction (LVNC): a clinical feature more often observed in terminal deletion 1p36 than previously expected. Eur J Med Genet 2008;51:685–8.
[36] Gajecka M, Saitta SC, Gentles AJ, Campbell L, Ciprero K, Geiger E, et al. Recurrent interstitial 1p36 deletions: evidence for germline mosaicism and complex rearrangement breakpoints. Am J Med Genet A 2010;152A:3074–83.
[37] Faivre L, Morichon-Delvallez N, Viot G, Martinovic J, Pinson MP, Aubry JP, et al. Prenatal detection of a 1p36 deletion in a fetus with multiple malformations and a review of the literature. Prenat Diagn 1999;19:49–53.
[38] Riegel M, Castellan C, Balmer D, Brecevic L, Schinzel A. Terminal deletion, del(1)(p36.3), detected through screening for terminal deletions in patients with unclassified malformation syndromes. Am J Med Genet 1999;82:249–53.
[39] Redon R, Rio M, Gregory SG, Cooper RA, Fiegler H, Sanlaville D, et al. Tiling path resolution mapping of constitutional 1p36 deletions by array-CGH: contiguous gene deletion or "deletion with positional effect" syndrome? J Med Genet 2005;42:166–71.
[40] Digilio MC, Bernardini L, Lepri F, Giuffrida MG, Guida V, Baban A, et al. Ebstein anomaly: genetic heterogeneity and association with microdeletions 1p36 and 8p23.1. Am J Med Genet A 2011;155A:2196–202.
[41] Sinkovec M, Kozelj M, Podnar T. Familial biventricular myocardial noncompaction associated with Ebstein's malformation. Int J Cardiol 2005;102:297–302.
[42] Sentex E, Wang X, Liu X, Lukas A, Dhalla NS. Expression of protein kinase C isoforms in cardiac hypertrophy and heart failure due to volume overload. Can J Physiol Pharmacol 2006;84:227–38.
[43] Wu SC, Solaro RJ. Protein kinase C zeta. A novel regulator of both phosphorylation and de-phosphorylation of cardiac sarcomeric proteins. J Biol Chem 2007;282:30691–8.
[44] Kim BJ, Zaveri HP, Shchelochkov OA, Yu Z, Hernandez-Garcia A, Seymour ML, et al. An allelic series of mice reveals a role for RERE in the development of multiple organs affected in chromosome 1p36 deletions. PLoS One 2013;8:e57460.
[45] Barron DJ, Kilby MD, Davies B, Wright JG, Jones TJ, Brawn WJ. Hypoplastic left heart syndrome. Lancet 2009;374:551–64.
[46] Grossfeld PD. The genetics of hypoplastic left heart syndrome. Cardiol Young 1999;9:627–32.
[47] Sanchez-Valle A, Pierpont ME, Potocki L. The severe end of the spectrum: hypoplastic left heart in Potocki–Lupski syndrome. Am J Med Genet A 2011;155A:363–6.
[48] Chen CP, Chern SR, Hsu CY, Lee CC, Lee MS, Wang W. Prenatal diagnosis of de novo partial trisomy 13q (13q22 → qter) and partial monosomy 8p (8p23.3 → pter) associated with holoprosencephaly, premaxillary agenesis, hexadactyly, and a hypoplastic left heart. Prenat Diagn 2005;25:334–6.
[49] Tsuyusaki Y, Yoshihashi H, Furuya N, Adachi M, Osaka H, Yamamoto K, et al. 1p36 deletion syndrome associated with Prader–Willi-like phenotype. Pediatr Int 2010;52:547–50.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2014) ••, ••–••

doi: 10.1111/ped.12257

Original Article

Epilepsy and West syndrome in neonates with hypoxic–ischemic encephalopathy

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Abstract ***Background***: Perinatal hypoxic–ischemic encephalopathy (HIE) has been linked to the development of late-onset seizures. The aim of the present study was to determine the incidence of epilepsy and West syndrome in children with perinatal HIE and identify factors associated with the development of postnatal seizure disorders.

Methods: We retrospectively enrolled 208 term and late-preterm infants diagnosed with perinatal HIE from April 2000 to March 2009 at Saitama Children's Medical Center. Children with obvious multiple anomalies and known chromosomal abnormalities were excluded. A questionnaire was distributed to parents to determine seizure-related outcomes. Medical records were retrospectively reviewed and relevant clinical parameters were analyzed.

Results: In total, 162 questionnaires were answered (77.9%). Of the 162 subjects, 26 (16.0%) developed epilepsy, and eight subjects (4.9%) were diagnosed with West syndrome. Neonatal seizures occurred in 72 subjects (44.4%). The incidence of epilepsy and West syndrome was significantly higher in infants who experienced neonatal seizures than in those without seizure history. A total of 82 subjects were diagnosed with moderate ($n = 52$) or severe HIE ($n = 30$), of whom 57 subjects (69.5%) received therapeutic hypothermia. The incidence of epilepsy was significantly lower in these treated subjects. In addition, subjects with moderate or severe HIE were significantly more likely to develop late-onset epilepsy and West syndrome than those with mild HIE.

Conclusions: The severity of perinatal HIE and neonatal seizures is a potential risk factor for the development of late-onset seizures. Therapeutic hypothermia may reduce the risk of the development of epilepsy in such cases.

Key words epilepsy, hypoxic–ischemic encephalopathy, neonatal seizure, therapeutic hypothermia, West syndrome.

Neurological sequelae of perinatal hypoxic–ischemic encephalopathy (HIE) are an important concern in neonatal intensive care units. Advances in the care of high-risk newborns have contributed to a decrease in the mortality rate, but neurological morbidity often increases in surviving neonates. The primary cause of HIE is perinatal asphyxia, and the incidence of perinatal HIE is approximately 1–2 per 1000 live term births.[1,2] Neonatal mortality occurs in 15–20% of newborns with perinatal HIE, while neurological handicaps such as cerebral palsy, developmental delay, and epilepsy develop in approximately 25% of survivors.[3] The rate of epilepsy developing in neonates diagnosed with perinatal HIE ranges from 9% to 33%.[4–6] A previous study reported a fivefold greater risk of developing epilepsy in children with a history of perinatal HIE than in those without, while mild and moderate HIE seemed unrelated to post-neonatal epilepsy. Furthermore, these authors found an association between post-neonatal epilepsy and moderate/severe abnormalities on neonatal magnetic resonance imaging in children with HIE, with diverse imaging findings.[4–6] Although perinatal HIE is a known risk factor for the development of epilepsy, few studies have examined the correlation between perinatal factors and the development of late-onset epilepsy in infants with perinatal HIE.

West syndrome is an intractable and age-dependent form of epilepsy characterized by the clinical triad of epileptic spasms, psychomotor developmental arrest, and hypsarrhythmia, and it is classified into cryptogenic and symptomatic types. Symptomatic West syndrome has a variety of etiologies that are usually categorized according to the time of cerebral injury as prenatal, perinatal, and postnatal. Perinatal factors account for 14–25% of all West syndrome cases.[7] Recently, an increase in the incidence of perinatal West syndrome was reported, and perinatal HIE was identified as the most common etiology of West syndrome in these studies.[8,9] Although children with perinatal HIE are known to be at risk of developing West syndrome, no study has reported the incidence of West syndrome in these children.

The aim of the present study was therefore to determine the incidence of late-onset epilepsy and West syndrome in children with perinatal HIE and to identify factors associated with the development of postnatal epilepsy.

Methods

In the present study, we retrospectively enrolled neonates who were admitted to the Neonatal Intensive Care Unit at Saitama

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Received 13 June 2013; revised 30 September 2013; accepted 25 October 2013.

Children's Medical Center, Saitama, Japan. Written informed consent to participate in this study was obtained from the parents of each subject, and the study protocol was approved by the ethics committee of the hospital.

Subjects were term and late-preterm infants (gestational age [GA] at birth, 34–41 weeks) who were diagnosed with perinatal HIE from April 2000 to March 2009 at Saitama Children's Medical Center and were >12 months old at the time of the study. Perinatal HIE was defined on the basis of the Sarnat clinical staging system, which consists of three stages based on physician assessment of several parameters, including consciousness, neuromuscular control, primitive reflex action, autonomic function, and seizure incidence.[10] All newborns were examined multiple times until discharged to confirm the diagnosis of HIE and exclude the presence of other neonatal diseases/disorders. Children who died and those with major brain anomalies or other major organ systems, known chromosomal abnormalities, evidence of congenital metabolic disease, and intrauterine infections were excluded.

A simple questionnaire was distributed to the parents of the subjects to determine seizure-related outcomes, which included items concerning the classification of epilepsy, frequency of seizures, medications, and age at seizure onset. The mean age of subjects at the time the questionnaire was distributed was 5.5 ± 2.5 years.

A trained neonatologist retrospectively extracted delivery data and clinical information, including GA at birth, birthweight, Apgar score, Sarnat clinical stage of perinatal HIE, history of neonatal seizure, hypoglycemia, apnea, and therapeutic hypothermia from the subjects' neonatal medical records. Neonatal seizures were diagnosed according to Volpe's classification by direct observation of clinical changes and/or observation of electroencephalogram changes.[11] Hypoglycemia was defined as blood sugar <40 mg/dL at first examination. Indications for therapeutic hypothermia were moderate or severe perinatal HIE according to the Sarnat clinical staging system, GA at birth >34 weeks, birthweight >2000 g, Apgar score ≤6 (at 5 min), and blood pH <7.0 or serum lactate ≥8 mmol/L. Exclusion criteria for therapeutic hypothermia treatment were age >6 h at birth, lack of informed parental consent, hypotension (mean blood pressure, <40 mmHg), bleeding tendency, persistent pulmonary hypertension, and severe infection.[12]

Data are presented as mean ± SD, and $P < 0.05$ was considered statistically significant. Fisher's exact test was used to compare categorical variables. Predictors of post-neonatal epilepsy and West syndrome were identified using multivariate logistic regression analysis with a forced-entry model. Data were analyzed using SPSS version 19 (SPSS, Chicago, IL, USA).

Results

A total of 287 newborns were diagnosed with perinatal HIE from April 2000 to March 2009 at Saitama Children's Medical Center. Of these, 79 were excluded because of death ($n = 38$), major brain anomalies or other major organ systems ($n = 24$), or chromosomal abnormalities ($n = 17$). Thus, 208 subjects were enrolled in the study. A total of 162 completed questionnaires (77.9%) were successfully obtained from the subjects' parents. Subject characteristics are listed in Table 1.

Table 1 HIE subject clinical characteristics ($n = 162$)

Perinatal characteristics	n (%) or mean ± SD
Male,	84 (52)
GA at birth (weeks)	38.7 ± 2.0
Birthweight (g)	2796 ± 527
Apgar score	
1 min	2.8 ± 1.9
5 min	4.2 ± 1.8
Sarnat clinical stages of perinatal HIE	
Mild	80 (49)
Moderate	52 (32)
Severe	30 (19)
Therapeutic hypothermia	57 (35)
Neonatal seizure	72 (44)
Hypoglycemia	27 (17)
<Apnea	17 (11)

GA, gestational age; HIE, hypoxic–ischemic encephalopathy.

Of the 162 subjects, 26 (16.0%) developed epilepsy, and eight subjects (4.9%) were diagnosed with West syndrome. The mean age at seizure onset was 17.4 ± 13.5 months in subjects who developed epilepsy and 5.1 ± 1.7 months in those who developed West syndrome. All subjects with epilepsy were receiving medical treatment at the time of the study. Neonatal seizures occurred in 72/162 subjects (44.4%), of which 18 (25%) developed epilepsy and seven (9.7%) developed West syndrome. Therefore, the incidence of epilepsy and West syndrome was significantly higher in neonates with a history of neonatal seizures than in those without ($P < 0.05$; Tables 2,3). Hypoglycemia and apnea were present in 27 (16.7%) and in 17 (10.5%) of the 162 subjects, respectively.

According to the Sarnat clinical staging system, 80 subjects were classified with mild HIE, 52 with moderate HIE, and 30 with severe HIE. To evaluate the correlation between perinatal HIE and the development of late-onset seizures, the 162 subjects were divided into the following two groups: those with mild perinatal HIE and those with moderate or severe perinatal HIE. Subjects with moderate or severe perinatal HIE were signifi-

Table 2 HIE subject characteristics vs presence of PNE

Subjects	With PNE	Without PNE	P
With moderate or severe HIE	21	61	
With mild HIE	5	75	0.002
With neonatal seizures	18	54	
Without neonatal seizures	8	82	0.010
With hypoglycemia	4	23	
Without hypoglycemia	22	113	0.924
With apnea	3	14	
Without apnea	23	122	0.873
With therapeutic hypothermia	11	46	
Without therapeutic hypothermia	10	15	0.048

HIE, hypoxic-ischemic encephalopathy; PNE, post-neonatal epilepsy.

Table 3 HIE subject characteristics vs presence of West syndrome

Subjects	With WS	Without WS	*P*
With moderate or severe HIE	8	74	
With mild HIE	0	80	0.012
With neonatal seizures	7	65	
Without neonatal seizures	1	89	0.032
With hypoglycemia	1	26	
Without hypoglycemia	7	128	0.871
With apnea	1	16	
Without apnea	7	138	0.688
With therapeutic hypothermia	4	,53	
Without therapeutic hypothermia	4	,21	0.207

HIE, hypoxic–ischemic encephalopathy; WS, West syndrome.

cantly more likely to develop late-onset epilepsy and West syndrome than those with mild perinatal HIE ($P < 0.05$; Tables 2,3).

Subjects with moderate or severe perinatal HIE were considered as candidates for therapeutic hypothermia at Saitama Children's Medical Center. Of the 82 subjects with moderate or severe perinatal HIE, 25 were not eligible for this treatment according to the exclusion criteria, which included bleeding tendency ($n = 1$), age >6 h, and lack of informed consent ($n = 24$). Therefore, therapeutic hypothermia was induced in only 57/82 subjects (69.5%). The incidence of epilepsy was significantly lower in these 57 subjects than in the remaining 25 subjects ($P = 0.048$; Table 2). No statistically significant correlation was found between the development of West syndrome and treatment with therapeutic hypothermia ($P = 0.207$; Table 3).

The association between postnatal epilepsy and factors associated with moderate or severe HIE persisted after multiple logistic regression analysis (odds ratio [OR], 7.469; 95% confidence interval [CI]: 2.007–27.800; Table 4). The effect of therapeutic hypothermia also persisted (OR, 0.343; 95%: 0.120–0.983; Table 4). The correlation between postnatal epilepsy and factors associated with neonatal seizure did not persist nor did the association between West syndrome and any other risk factors.

Discussion

Epilepsy is one of the most important causes of neurological sequelae in perinatal HIE. The objectives of this study were to determine the incidence of epilepsy and West syndrome in children with perinatal HIE and identify factors associated with postnatal epilepsy. This retrospective cohort study included 162 subjects with HIE, 26 (16.0%) of whom developed epilepsy. The incidence of perinatal HIE-associated epilepsy in the present study was similar to that reported in previous studies (9%–33%).[4–6]

Table 4 Significant factors for onset of PNE

	OR	95%CI	*P*
Moderate or severe HIE (vs with mild HIE)	7.469	2.007–27.800	0.003
Neonatal seizures (vs without)	1.889	0.654–5.461	0.240
Hypoglycemia (vs without)	1.018	0.296–3.501	0.977
Apnea (vs without)	1.198	0.281–5.116	0.807
Therapeutic hypothermia (vs without)	0.343<	0.120–0.983	0.046

CI, confidence interval; OR, odds ratio; PNE, post-neonatal epilepsy.

Previous studies reported an incidence of 3–5 West syndrome cases per 10 000 live births, and that age at onset of West syndrome was 3–12 months (peak, 5 months) in 90% of cases.[7,13] Of the 162 perinatal subjects with HIE in the present study, eight (4.9%) developed West syndrome, and the age at onset of spasm was 5.1 ± 1.7 months. Considering that the incidence of perinatal HIE is 10–20 per 10 000 live term births and that perinatal HIE is the cause of West syndrome in 14–25% of cases, the incidence of 4.9% in subjects with perinatal HIE in the present study was considered reasonable.

In the present study, 72/162 subjects (44.4%) had a history of neonatal seizures. This result supports those of previous studies, which reported that perinatal HIE was the most frequent cause of neonatal seizures and accounted for 43.3–60.0% of neonatal seizures.[4,14,15] Few studies have investigated the correlation between the incidence of neonatal seizures and development of epilepsy. Neonatal seizure was identified as a risk factor in a study by Pisani *et al.*, although its effect was not significant after adjusting for the degree of HIE.[4] Glass *et al.* reported that neonatal status epilepticus was a highly significant risk factor for the development of epilepsy.[16] In the present study, the incidences of epilepsy and West syndrome were significantly higher in subjects with a history of neonatal seizures than in those without ($P < 0.05$). Nevertheless, the correlation between postnatal epilepsy/West syndrome and factors associated with neonatal seizure did not persist after multiple logistic regression analysis. Neonatal seizure may cause brain injury through impaired cerebral metabolism and secondary induced epilepsy,[17–19] but its effect is controversial after adjusting for the degree of HIE.

Therapeutic hypothermia is a recognized treatment for perinatal HIE, and it is useful for decreasing the mortality rate and preventing neurodevelopmental disabilities. Azzopardi *et al.* reported that therapeutic hypothermia decreased the risk of cerebral palsy and improved developmental and gross motor function scores in subjects assessed at approximately 18 months of age, although it was not significantly associated with other neurological outcomes, including seizures.[20] In the present study, however, the incidence of epilepsy was significantly lower in subjects who received therapeutic hypothermia than in those who were not thus treated. The mean age of subjects assessed was 5.5 ± 2.5 years, and the longer observation period in the present study compared with previous studies may account for the different findings.

The present study determined the incidence of epilepsy and West syndrome in subjects with perinatal HIE to be 16.0% and 4.9%, respectively, with the severity of perinatal HIE and neonatal seizures identified as potentially important risk factors for the development of late-onset epilepsy and West syndrome. Nevertheless, the correlation between postnatal epilepsy/West syndrome

and neonatal seizures was not identified on multiple logistic regression analysis. Thus, therapeutic hypothermia may reduce the risk of developing epilepsy, but many issues remain concerning newborns with perinatal HIE and further studies with a larger number of subjects will be necessary to resolve these unanswered issues.

References

1 Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum. Dev.* 2010; **86**: 329–38.
2 Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: Incidence, clinical course and outcome in a Swedish population. *Acta Paediatr.* 1995; **84**: 927–32.
3 Volpe J. *Neurology of the Newborn*, 5th edn. WB Saunders, Philadelphia, 2008.
4 Pisani F, Orsini M, Braibanti S, Copioli C, Sisti L, Turco EC. Development of epilepsy in newborns with moderate hypoxic-ischemic encephalopathy and neonatal seizures. *Brain Dev.* 2009; **31**: 64–8.
5 Belet N, Belet U, Incesu L *et al.* Hypoxic-ischemic encephalopathy: Correlation of serial MRI and outcome. *Pediatr. Neurol.* 2004; **31**: 267–74.
6 van Kooij BJ, van Handel M, Nievelstein RA, Groenendaal F, Jongmans MJ, de Vries LS. Serial MRI and neurodevelopmental outcome in 9- to 10-year-old children with neonatal encephalopathy. *J. Pediatr.* 2010; **157**: 221–227.
7 Riikonen R. Decreasing perinatal mortality: Unchanged infantile spasms morbidity. *Dev. Med. Child Neurol.* 1995; **37**: 232–8.
8 Watanabe K. West syndrome: Etiological and prognostic aspects. *Brain Dev.* 1998; **20**: 1–8.
9 Osborne JP, Lux AL, Edwards SW *et al.* The underlying etiology of infantile spasms (West syndrome): Information from the United Kingdom Infantile Spasms Study (UKISS) on contemporary causes and their classification. *Epilepsia* 2010; **51**: 2168–74.
10 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch. Neurol.* 1976; **10**: 696–705.
11 Volpe JJ. Neonatal seizures: Current concepts and revised classification. *Pediatrics* 1989; **84**: 422–8.
12 Shimizu M. Topics of the brain protection in neonate, which has been your better choice, the brain hypothermia or the medicine? *No To Hattatsu* 2009; **41**: 111–17 (in Japanese).
13 Panayiotopoulos CP. *A Clinical Guide to Epileptic Syndromes and Their Treatment*, 2nd edn. Springer, New York, 2010.
14 Andre M, Matisse N, Vert P, Debruille C. Neonatal seizures: Recent aspects. *Neuropediatrics* 1988; **19**: 201–7.
15 Verrotti A, Domizio S, Sabatino G, Morgese G. Early infantile epileptic encephalopathy: A long-term follow-up study. *Childs Nerv. Syst.* 1996; **12**: 530–33.
16 Glass HC, Hong KJ, Rogers EE *et al.* Risk factors for epilepsy in children with neonatal encephalopathy. *Pediatr. Res.* 2011; **70**: 535–40.
17 Leuthner SR, Das UG. Low Apgar scores and the definition of birth asphyxia. *Pediatr. Clin. North Am.* 2004; **51**: 737–45.
18 Younkin DP, Delivoria-Papadopoulos M, Maris J, Donlon E, Clancy R, Chance B. Cerebral metabolic effects of neonatal seizures measured with in vivo 31P NMR spectroscopy. *Ann. Neurol.* 1986; **20**: 513–19.
19 Murray DM, Ryan CA, Boylan GB, Fitzgerald AP, Connolly S. Prediction of seizures in asphyxiated neonates: Correlation with continuous video-electroencephalographic monitoring. *Pediatrics* 2006; **118**: 41–6.
20 Azzopardi DV, Strohm B, Edwards AD *et al.* TOBY Study Group. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N. Engl. J. Med.* 2009; **361**: 1349–58.

FULL-LENGTH ORIGINAL RESEARCH

Long-term course of Dravet syndrome: A study from an epilepsy center in Japan

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Epilepsia, **(*):1–11, 2014
doi: 10.1111/epi.12532

Summary

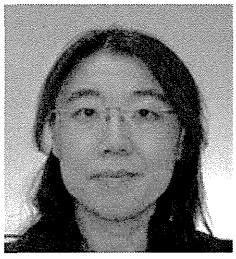
Objective: This study attempted to clarify the long-term course of Dravet syndrome (DS).

Methods: Sixty-four patients diagnosed with DS (44 with typical DS, and 20 with atypical DS) were studied. The long-term outcomes of clinical seizures, electroencephalographic findings, neuropsychological findings, and social situation were analyzed. The follow-up period ranged from 11 to 34 years 5 months (median 24 years).

Results: At the last visit, the ages ranged from 19 years to 45 years (median 30 years). Fifty-nine patients continued to have generalized tonic–clonic seizures (GTCS). Status epilepticus and unilateral seizures were not observed and myoclonic seizures, atypical absence seizures, and photosensitive seizures were resolved in most patients. The frequency of complex partial seizures was equally low, with five patients at presentation and six patients at the last visit, respectively. Five patients achieved seizure remission (seizure-free for 1 year or longer). Only 1 of 44 patients with typical DS had seizure remission, whereas 4 of 20 patients with atypical DS remitted, with a statistically significant difference between the two phenotypes ($p = 0.03$). Intellectual disability was found in all patients; especially, severe intellectual disability was prevalent. Patients with atypical DS tended to have milder intellectual disability compared to those with typical DS ($p = 0.0283$). Occipital alpha rhythm in the basic activity was associated with milder intellectual disability ($p = 0.0085$). The freedom from seizures correlated with appearance of occipital alpha rhythms ($p = 0.0008$) and disappearance of epileptic discharges ($p = 0.0004$). Two patients with GTCS died. Mutations of the neuronal voltage-gated sodium channel alpha subunit type 1 gene were detected at a high frequency (33 of 36 patients examined). Seizure remission was found only in the missense mutation group.

Significance: The long-term seizure and intellectual outcomes are extremely poor in patients with typical DS compared to those with atypical DS. Epilepsy phenotype may influence long-term course of DS.

KEY WORDS: Severe myoclonic epilepsy in infancy, Intractable childhood epilepsy with generalized tonic–clonic seizure, Neuronal voltage-gated sodium channel alpha subunit type 1 gene, Long-term course.



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Dravet syndrome (DS), otherwise known as severe myoclonic epilepsy in infancy (SMEI), is a rare epileptic syndrome estimated to affect one in 40,000 children.[1] A recent report from the United Kingdom estimated the incidence of mutation-positive DS to be one in 40,900 births.[2] Although DS is representative of epileptic encephalopathy,[3] in which

Accepted November 30, 2013.

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seizures remain refractory to all conventional antiepileptic drugs (AEDs), the long-term clinical course of this epileptic syndrome is largely unknown.

DS presents in the first year of life in an otherwise normal infant in the form of febrile or afebrile generalized or unilateral clonic seizures, or generalized tonic–clonic seizures (GTCS), later on with additional myoclonic seizures, atypical absence seizures, or partial seizures. The seizures are refractory to AEDs, and mental retardation appears from the second year,[4] but may occur later up to the fourth year.[5] The term "borderline SMEI" (SMEB) has been used to designate a subset of patients with similar clinical symptoms but who do not manifest minor seizures such as myoclonic seizures and atypical absence seizures. We advocated the term "intractable childhood epilepsy with generalized tonic–clonic seizure" (ICEGTC) for a group of patients who manifest refractory grand mal seizures with/without complex partial seizures (CPS).[6,7] ICEGTC overlaps with SMEB in many aspects.

Because the older terminologies such as SMEI, SMEB, and ICEGTC are somewhat confusing, in this article we use the term "typical DS" for core SMEI and "atypical DS" for ICEGTC and SMEB. Atypical DS refers to patients without minor generalized seizures.

Mutations of the neuronal voltage-gated sodium channel alpha subunit type 1 gene (*SCN1A*) were first reported in core SMEI (typical DS) patients.[8] The majority of patients with DS have mutations in the *SCN1A*.[9–11] Currently, these related epilepsies are known to have high frequencies of *SCN1A* mutations; they are positioned as the SMIE spectrum, and included in DS.[11,12]

DS is one form of epileptic encephalopathy, but the long-term clinical course is not well studied. Only four series included patients older than 20 years were published.[13–16] Although reports of various investigators concur on the point that the course of disease is marked by uncontrolled seizures, it remains unclear whether seizure control is ultimately achieved in the long term, whether there is a relationship between seizure severity and intellectual outcome, and what is the social and survival outcome of these patients. Fujiwara et al.[17] studied the clinical course of 29 patients with DS aged from 6 years 7 months to 17 years 10 months and found no seizure remission in all the patients, regardless of phenotype. The present study is an extension of the previous study, with a larger number of cases and prolonged observation period, aiming to clarify the long-term outcomes in these patients.

Methods and Subjects

We performed a retrospective review of clinical records to identify patients with DS who presented to our epilepsy center before 1998 and were followed for at least 10 years and who were older than 19 years at the last visit. In this study, patients with a diagnosis of SMEI or ICEGTC who had the following characteristics were selected. (1) Before onset, the infant had no history of brain damage and no developmental problem. (2) Around 6 months after birth, fever or bathing triggered the first episode of unilateral or bilateral clonic or tonic–clonic seizure. (3) The first seizure may manifest as convulsive status. (4) Unilaterally dominant seizure may involve the left side or right side alternatively. (5) Uncontrolled convulsive seizures persisted, with additional appearance of myoclonic seizures or atypical absence seizures after 1 year of age. (6) Cases without the above minor generalized seizures were also included. (7) Complex partial seizures may coexist. The above characteristics match the description of DS in a recent review.[18] Seizure remission was defined as seizure free for 1 year or longer at last visit. The Fisher's exact test and chi-square were used for statistical analysis. A p-value of <0.05 was regarded as significant.

A total of 64 patients (30 male and 34 female) were identified based on the above criteria. Data of clinical seizures, neuropsychiatric disorders, and social outcome were extracted. All the patients had experienced hospitalization for treatment, and thereafter were followed and treated at the outpatient clinic. All the patients underwent electroencephalography (EEG) examination at the last visit. During follow-up, EEG including awake and sleep states was examined at least once a year in almost all cases. Intellectual level was determined by clinical assessment. *SCN1A* analysis was conducted in 36 patients, and gene abnormalities were detected in 33 patients. The methods of gene analysis were described elsewhere.[9,11] Written informed consent was obtained from the parents or responsible adults where necessary, and the study protocol was approved by the ethical committees of Shizuoka Institute of Epilepsy and Neurological Disorders and of the Institutional Review Board of RIKEN-BSI.

Results

Patients

The ages of epilepsy onset ranged from 2 to 11 months (median 5 months). The ages of initial presentation to our epilepsy center ranged from 1 year to 16 years and 8 months (median 5 years and 5 months); more than half of the patients (33/64, 51.6%) were aged 5 years or below. The ages at the last visit ranged from 19 years to 45 years (median 30 years). The follow-up period ranged from 11 years to 34 years and 5 months (median 24 years). A family history of febrile convulsion was found in 29 patients, and a family history of epilepsy was found in 8 patients. All patients were taking multiple AEDs including carbamazepine in three patients, but no new drugs such as stiripentol, topiramate, or lamotrigine. Discontinuation of AEDs was not attempted in any of the patients.

The 64 patients were divided into two groups according to the presence or absence of "combined" minor generalized seizures (myoclonic seizures or atypical absence seizures)

other than GTCS throughout the course of epilepsy. Forty-four patients who had minor generalized seizures met the criteria of typical DS according to the proposal of the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE).[19] Twenty patients were classified as atypical DS.

Seizure outcome

From onset to presentation

In all patients, prolonged generalized clonic seizures, GTCS, or unilateral seizures (US) were often induced by fever or bathing. These seizures occurred in clusters and often evolved to status epilepticus (SE). Despite treatment with AEDs, these seizures, whether febrile or afebrile, persisted uncontrolled, and convulsive SE occurred repeatedly in 39 patients. Myoclonic seizures coexisted in 40 patients, atypical absence seizures in 24 patients, and CPS in 18 patients (some patients had multiple seizure types). Photosensitive and/or pattern-sensitive seizures were found in seven patients.

At presentation and last visit

At presentation to our epilepsy center, all patients had GTCS or US. Eleven patients had recurrent SE, 24 had myoclonic seizures, 19 had atypical absence seizures, and five had CPS (some patients had multiple seizure types). Photosensitive and/or pattern-sensitive seizures were present in seven patients.

At the last visit, none of the patients had US and SE no longer occurred; 59 patients had GTCS, 5 had myoclonic seizures, 1 had atypical absence seizures, and 6 had CPS. Photo-sensitive and/or pattern-sensitive seizures were observed in no patients. In the majority of the patients, GTCS persisted, but the seizure frequency tended to decrease over time. Low-grade fever became less provocative. In most cases, frequency of GTCS was between weekly and monthly occurrence. Seizures occurred mostly during nocturnal sleep. There was an increase in the proportion of patients with GTCS reduced to yearly occurrence. In five patients, GTCS were controlled at ages ranging from 15 to 28 years (median 19 years). All five cases were terminal remission cases and were free from all types of seizure for 5–22 years at the time of the last visit (Figs. 1 and 2).

When examining the seizure remission rate according to phenotype, only one (2.3%) of 44 patients with typical DS had seizure remission, whereas 4 (20.0%) of 20 patients with atypical DS remitted, with a significant difference between the two phenotypes, indicating an association between seizure remission rate and phenotype in patients with DS ($p = 0.03$).

EEG

At presentation to our epilepsy center, 48 patients (75.0%) had epileptic discharges of various qualities. The

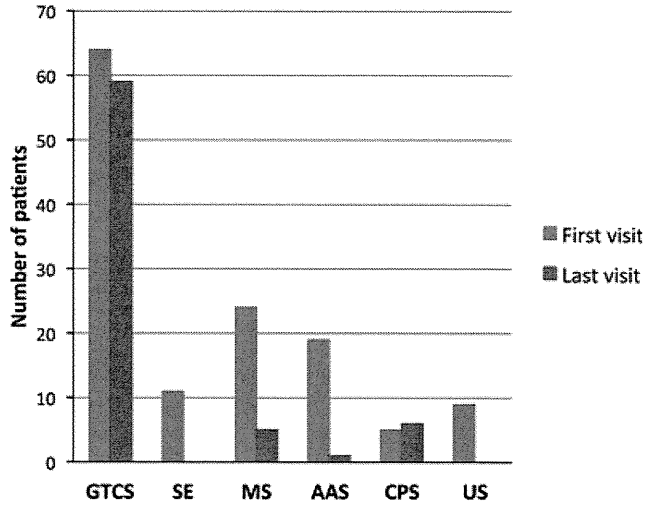

Figure 1.
Evolution of various seizure types in patients with DS. At the last visit, none of the patients had US, and none had SE. MS and AAS decreased with time. The frequency of patients manifesting CPS was unchanged. In the majority of the patients, GTCS persisted. GTCS, generalized tonic–clonic seizures; SE, status epilepticus; MS, myoclonic seizures; AAS, atypical absence seizures; CPS, complex partial seizures; US, unilateral seizures; DS, Dravet syndrome.
Epilepsia © ILAE

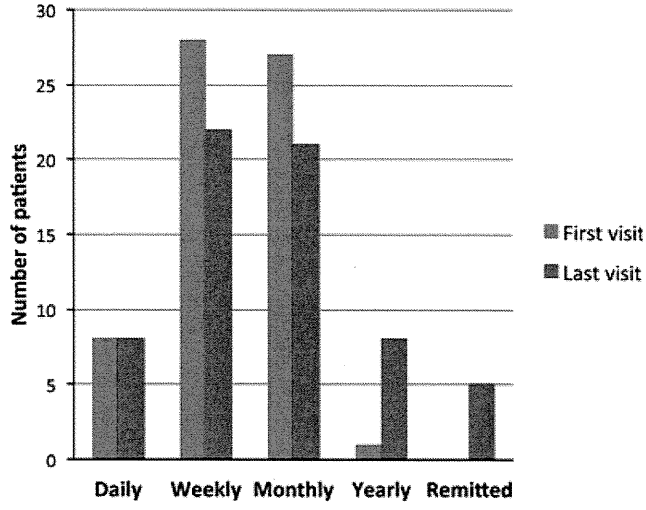

Figure 2.
Evolution of GTCS frequency. The seizure frequency tended to decrease over time. There was a marked increase in the proportion of patients with GTCS reduced to yearly occurrence. In five patients, GTCS were completely controlled. GTCS, generalized tonic–clonic seizures.
Epilepsia © ILAE

epileptic discharges consisted of diffuse spike-wave complex or diffuse polyspike-wave complex in 14 patients (21.9%); diffuse spike-wave complex, diffuse polyspike-wave complex and focal spike and sharp waves in 14 patients (21.9%); focal spike and sharp waves in 20 patients

(31.2%; frontal in three patients, temporal in 5 patients, and multifocal in 12 patients). When the patients were divided into those with diffuse epileptic discharges and those with focal epileptic discharges, the distribution was similar, with 28 patients (43.8%) in the former group and 34 patients (53.1%) in the latter group (some patients had both types). Epileptic discharges were not observed in 16 patients (25.0%) (Fig. 3).

At the last visit, epileptic discharges were detected in 49 patients (76.6%). The epileptic discharges consisted of diffuse spike-wave complex or diffuse polyspike-wave complex in 5 patients (7.8%); diffuse spike-wave complex, diffuse polyspike-wave complex and focal spike and sharp waves in 1 patient (1.6%); and focal spike and sharp waves in 43 patients (67.2%; frontal in 21 patients, temporal in 3 patients, central in 1 patient, parietal in 1 patient, occipital in 1 patient, and multifocal in 16 patients). When the patients were divided into those with diffuse epileptic discharges and those with focal epileptic discharges, there were 6 patients (9.4%) in the former group and 44 patients (68.8%) in the latter group, showing a marked dominance of focal epileptic discharges (some patients had both types). Epileptic discharges were not observed in 15 patients (23.4%). Of these, five patients achieved freedom from seizures. On the other hand, among 49 patients who had epileptic discharges at the last visit, no patients achieved freedom from seizures. The freedom from seizures significantly correlated with disappearance of epileptic discharges ($p = 0.0004$). Among the 16 patients who had no epileptic discharges at presentation (from 1 to 16 years of age), 11 patients had epileptic discharges at the last visit. Of the remaining five patients with no epileptic discharges at the last visit, four patients had rare epileptic discharges sometimes during follow-up. In one patient there were no epileptic discharges at all despite numerous EEG recordings during follow-up (7 years 10 months to 34 years of age).

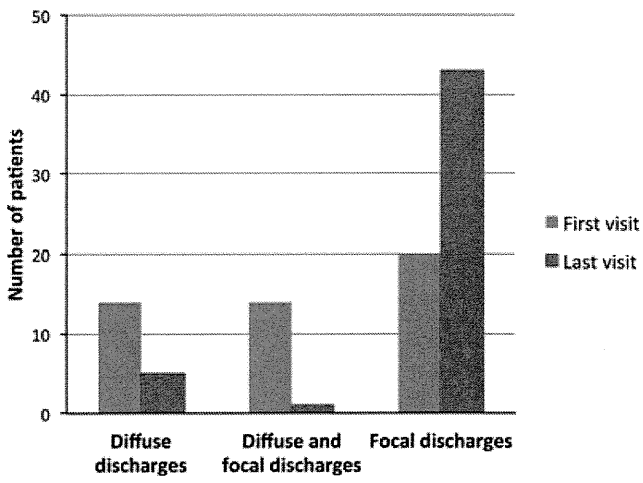

Figure 3.
Evolution of EEG findings. Focal discharges became dominant during follow-up.
Epilepsia © ILAE

Diffuse high potential slow wave activity with central and parietal dominance was observed in 49 patients at the time of presentation to our epilepsy center, but the number was reduced to 16 at their last visit. At the last visit, occipital alpha rhythms were found in their basic EEG activity in 17 patients. Among them, 3 patients had slower posterior dominant rhythms (PDRs) at 8 Hz and 14 patients had normal PDRs ranging from 9 to 11 Hz. In five patients who were taking benzodiazepine, β waves were found accompanied by PDRs. Among 17 patients who had occipital alpha rhythms, 5 patients achieved freedom from seizures. On the other hand, among 47 patients who had no occipital alpha rhythms no patients achieved freedom from seizures. The freedom from seizures significantly correlated with appearance of occipital alpha rhythms in the background activity ($p = 0.0008$).

Regarding correlation between occipital alpha rhythms and epileptic discharges at the last follow-up EEG, 17 patients had occipital alpha rhythms; of these, 7 had epileptic discharges and 10 had no epileptic discharges. Forty-seven patients had no occipital alpha rhythms; of these 40 had epileptic discharges and 7 had no epileptic discharges. The appearance of occipital alpha rhythms correlated significantly with the disappearance of epileptic discharges ($p = 0.001$; Table 1).

Neuropsychological outcome at the last visit

Four patients were bedridden; whereas the remaining 60 patients were capable of walking, over half of them had ataxic gait. Four patients had hemiplegia, all of whom had permanent hemiplegia as a sequel of prolonged febrile SE, which occurred between 1 year and 1 month and 4 years of age.

Intellectual disability was observed in all patients: severe in 49 patients (76.5%), moderate in 12 patients (18.8%), and mild in 3 patients (4.7%), with most patients having severe intellectual disability. Seventeen patients (26.6%) were admitted to institution, 45 patients (70.3%) were attending

Table 1. Occipital alpha rhythms and outcome

Occipital alpha rhythms at last visit (n)	Patients with seizure freedom (n)[a]	Patients without epileptic discharges (n)[b]	Patients with mild intellectual disability (n)[c]
+ (17)	5/17	10/17	3/17
− (47)	0/47	7/47	0/47

n, number of patients.
[a]The freedom from seizures correlated significantly with the appearance of occipital alpha rhythms ($p = 0.0008$).
[b]The appearance of occipital alpha rhythms correlated significantly with disappearance of epileptic discharges ($p = 0.001$).
[c]Patients with occipital alpha rhythms tended to have milder intellectual disability compared to those without ($p = 0.0085$).

sheltered workshops, and only two patients (3.1%) were living independently.

Table 2 shows the relationship between the severity of intellectual disability and the frequency of GTCS at the last visit. Among those with severe intellectual disability, 26 patients had daily to weekly GTCS, 21 patients had monthly to yearly GTCS, and 2 patients were seizure-free. Among those with moderate intellectual disability, four patients had daily to weekly GTCS, seven patients had monthly to yearly GTCS, and one patient was seizure-free. Among those with mild intellectual disability, no patient had daily to weekly GTCS, one patient had monthly to yearly GTCS, and two patients were seizure-free. These data showed a tendency of more severe intellectual disability in patients with higher GTCS frequency. A significant difference was observed between the severity of intellectual disability and the GTCS frequency at the last visit ($p = 0.0019$).

Among 17 patients showing occipital alpha rhythms, intellectual disability was mild in 3 patients, moderate in 4 patients, and severe in 10 patients. On the other hand, among 47 patients showing no occipital alpha rhythms, no patient had mild intellectual disability, whereas 8 patients had moderate and 39 patients had severe intellectual disability. Patients with occipital alpha rhythms tended to have milder intellectual disability compared to those without ($p = 0.0085$; Table 1).

When examining the cognitive outcome according to phenotype, among 44 patients with typical DS, intellectual disability was moderate in 8 patients and severe in 36 patients. No patient had mild intellectual disability. On the other hand, among 20 patients with atypical DS, 3 patients had mild, 4 patients had moderate, and 13 patients had severe intellectual disability. Patients with atypical DS tended to have milder intellectual disability compared to those with typical DS ($p = 0.0283$; Fig. 4).

Mortality

Among 64 patients, 2 patients (one male and one female) died. The causes of death were febrile illness–related sudden death in one patient and drowning in another. Both patients had GTCS, and one patient had myoclonic seizures and atypical absences. There was no significant correlation between phenotype and mortality.

Intellectual disability was severe in both patients. Both patients had persistent GTCS, and seizure frequency did not change before death.

Table 2. Intellectual outcome and frequency of generalized tonic–clonic seizures at the last visit

Intellectual disability	Daily to weekly seizures (n)	Monthly to yearly seizures (n)	Remitted (n)
Mild	0	1	2
Moderate	4	7	1
Severe	26	21	2

n, number of patients.
Higher GTCS frequency correlates with more severe intellectual disability ($p = 0.0019$).

SCN1A mutation and clinical course

Mutational analysis was performed on all coding exons and splice sites of *SCN1A* in 36 patients, and 32 heterozygous mutations were found in 33 patients (91.7%), consisting of 7 frameshift mutations in 7 patients, 7 nonsense mutations in 8 patients, and 18 missense mutations in 18 patients, which were not found in the control population ($n = 93–111$). No mutation was detected in the coding region of *SCN1A* in three patients (no. 34, 35, and 36). The mutations were all different except in one pair of monozygotic twins (Table 3).

Mutations of the *SCINA* were detected at a high frequency both in typical DS and in atypical DS (23/25; 92.0% vs. 10/11; 90.9%). Although statistically not significant, truncating mutations were more common in typical DS than in atypical DS (12/23; 52.2% vs. 3/10; 30.0%); missense mutations were less common in typical DS than in atypical DS (11/23; 47.8% vs. 7/10; 70.0%).

Regarding types of mutations, the patients were divided into two groups: 18 patients with missense mutation as group A, and 15 patients with truncating mutation (by grouping nonsense and frameshift mutations together) as group B. The two groups were compared with respect to clinical parameters and long-term outcomes. Group A consisted of 11 patients with typical DS and 7 patients with atypical DS, whereas group B consisted of 12 patients with typical DS and 3 patients with atypical DS (Fig. 5). Group A consisted of 8 male and 10 female patients, whereas group B consisted of seven male and eight female patients. The ages of onset ranged from 2 to 11 months (median 5 months) in group A, and 4 to 11 months (median 6 months) in group B. The ages at presentation to our center ranged from 1 year to 11 years and 2 months (median 4 years 5 months) in group A, and 1 year 8 months to 16 years 8 months (median 4 years 1 month) in group B. The ages of last visit ranged from 21 to 37 years (median 32 years) in group A, and 19 to 45 years (median 29 years) in group B.

When the clinical subtypes were compared, typical DS tended to be more common in group B, although the difference was not significant ($p = 0.2828$; Fig. 5). No differences in gender, and in ages at onset, at presentation, and at the last visit were found between group A and group B. The outcome of various seizure types was also not different between the two groups (Fig. 6). Three patients in group A were seizure-free for 1 year or longer. Complete seizure control was observed only in group A. The phenotypes in the remitted cases were typical DS in one patient; and atypical DS in two patients. At the last visit, intellectual disability

Table 3. Genotype-phenotype correlation

			Last visit						*SCN1A*	
Patient	Gender	DS phenotype	Age (years)	Seizure type	Intellectual disability	Motor disability	Social outcome	Alpha rhythms	Mutation	DNA Protein
1[11]	F	Typical	21	GTCS(w)	Severe	Ataxic gait	Institutionalized	–	Missense	c.5459T>C F1820S
2[11]	F	Typical	23	GTCS(m)	Severe	Unremarkable	Sheltered workshop	–	Missense	c.2845A>G M949V
3[11]	M	Typical	26	GTCS(w), MS	Severe	Ataxic gait	Institutionalized	–	Missense	c.5021C>A A1674D
4[11]	F	Typical	32	GTCS(m)	Severe	Unremarkable	Institutionalized	–	Missense	c.2921A>T N974I
5[11]	M	Typical	33	GTCS(w), CPS	Severe	Ataxic gait	Sheltered workshop	–	Missense	c.1028G>A G343E
6[11]	M	Typical	34	GTCS(m)	Severe	Ataxic gait	Institutionalized	+	Missense	W1801G
7[11]	F	Typical	34	Remitted	Severe	Ataxic gait	Sheltered workshop	+	Missense	c.3756C>G F1252L
8[11]	F	Typical	35	GTCS(m)	Severe	Ataxic gait	Sheltered workshop	+	Missense	c.307A>G S103G
9[35]	M	Typical	27	GTCS(m)	Severe	Unremarkable	Sheltered workshop	–	Missense	c.2957T>C L986P
10	F	Typical	32	GTCS(w)	Severe	Ataxic gait	Sheltered workshop	–	Missense	c.840G>C W280C
11	M	Typical	37	GTCS(m)	Severe	Right hemiplegia following febrile SE at 3 years 3 months old	Sheltered workshop	+	Missense	c.1055T>G V352G
12[11]	M	Atypical	27	GTCS(m)	Severe	Ataxic gait	Institutionalized	–	Missense	c.2902G>A G968R
13[36]	F	Atypical	28	Remitted	Mild	Unremarkable	Sheltered workshop	+	Missense	c.4096G>A V1366I
14[11]	M	Atypical	29	GTCS(d)	Severe	Ataxic gait	Sheltered workshop	–	Missense	c.2915T>C V972A
15[11]	M	Atypical	33	GTCS(m)	Severe	Ataxic gait	Sheltered workshop	–	Missense	c.5093C>T T1698I
16[11]	F	Atypical	33	Remitted	Severe	Unremarkable	Sheltered workshop	+	Missense	c.5389T>C F1797L
17[37]	F	Atypical	34	GTCS(m)	Mild	Unremarkable	Living independently	+	Missense	c.1177C>T R393C
18	F	Atypical	20	GTCS(w), CPS	Severe	Unremarkable	Sheltered workshop	–	Missense	c.719T>C L240P
19[11,38]	M	Typical	19	GTCS(w)	Severe	Ataxic gait	Institutionalized	–	Nonsense	c.3604C>T R1202X
20[11,38]	M	Typical	19	GTCS(w)	Severe	Ataxic gait	Institutionalized	–	Nonsense	c.3604C>T R1202X
21[9]	F	Typical	29	GTCS(w)	Severe	Ataxic gait	Sheltered workshop	–	Nonsense	c.4514C>A S1505X
22[11]	M	Typical	29	GTCS(d)	Severe	Ataxic gait	Sheltered workshop	+	Nonsense	c.3819G>A W1273X
23[9]	M	Typical	32	GTCS(y)	Severe	Ataxic gait	Sheltered workshop	–	Nonsense	c.2101C>T R701X
24[39]	F	Typical	24	GTCS(d)	Severe	Right hemiplegia following febrile SE at 2 years old	Institutionalized	–	Nonsense	c.1834>T R612X
25[35]	F	Atypical	26	GTCS(m)	Severe	Ataxic gait	Sheltered workshop	+	Nonsense	c.1738C>T R580X
26	F	Atypical	45	GTCS(y)	Severe	Left hemiplegia following febrile	Institutionalized	–	Nonsense	c.4219C>T R1407X

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