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Fortuitous Detection of a Submicroscopic Deletion at 1q25 in a Girl With Cornelia-de Lange Syndrome Carrying t(5;13)(p13.1;q12.1) by Array-Based Comparative Genomic Hybridization

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We report on a 2-year-old Japanese girl with Cornelia-de Lange syndrome (CdLS) who had mental and growth retardation, together with characteristic facial anomalies and mild extremity malformations. She had a balanced chromosomal translocation, 46,XX,t(5;13)(p13.1;q12.1) de novo. Surprisingly, this was the same translocation that had provided a clue to the identification of a major causative gene for CdLS, *NIPBL* [Krantz et al., 2004; Tonkin et al., 2004]. Using fluorescence in situ hybridization (FISH), the breakpoint was confirmed to lie within *NIPBL* at 5p13.1. Furthermore, array-based comparative genomic hybridization (array-CGH) demonstrated a cryptic 1-Mb deletion harboring six known genes at 1q25–q31.1. A FISH analysis of her parents confirmed that the deletion was de novo. Although patients with interstitial deletions at 1q are rare, some of their features were similar to those observed in our patient, indicating that her clinical manifestations are likely to be affected by not only the disruption of *NIPBL* but also the concomitant microdeletion at 1q25–q31.1. The present case suggests that array-CGH can uncover cryptic genomic aberrations affecting atypical phenotypes even in well-known congenital disorders. © 2007 Wiley-Liss, Inc.

Key words: Cornelia-de Lange syndrome; t(5 ;13)(p13.1; q12.1); 1q25 interstitial deletion; array-CGH

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INTRODUCTION

Cornelia-de Lange syndrome (CdLS [OMIM 122470]), first recognized as a distinct entity over 70 years ago, is a clinically variable developmental disorder characterized by facial dysmorphia, upper-extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities [Brachmann, 1916; de Lange, 1933]. The clinical features seen in individuals with classic CdLS are striking and easily recognizable, although there is marked variability, and a milder phenotype has been described [Ireland et al., 1993; Saul et al.,

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1993; Selicorni et al., 1993; Van Allen et al., 1993]. Indeed, even the first reported descriptions of CdLS were markedly discrepant in phenotype; Brachmann [1916] described major upper-limb-deficiency abnormalities, whereas de Lange [1933] reported no limb defects. This phenotypic variability and the lack of a diagnostic marker have made the diagnosis of and genetic counseling for CdLS challenging.

Recently, two independent groups identified the *NIPBL* gene as responsible for CdLS based on a breakpoint analysis of a balanced chromosomal translocation, t(5;13)(p13.1;q12.1) in the same patient with CdLS [Krantz et al., 2004; Tonkin et al., 2004], and subsequently, several types of *NIPBL* mutations including missense, splice site, nonsense, and frameshift mutations have been identified in patients with CdLS [Borck et al., 2004; Gillis et al., 2004; Krantz et al., 2004; Tonkin et al., 2004; Bhuiyan et al., 2006; Miyake et al., 2005; Yan et al., 2006].

Here, we report on a girl with CdLS, who has a de novo balanced translocation, t(5;13)(p13.1;q12.1). Our molecular cytogenetic analyses revealed that *NIPBL* was disrupted at the 5p13.1 breakpoint. Furthermore, array-based comparative genomic hybridization (array-CGH) and fluorescence in situ hybridization (FISH) revealed a de novo additional 1-Mb hemizygous deletion at 1q25–q31.1. In the present case, some atypical features of CdLS were likely to be affected by the cryptic 1q25–q31.1 interstitial deletion.

MATERIALS AND METHODS

Clinical Report

The subject is a 2-year-old Japanese girl with developmental disability, facial anomalies, and severe mental retardation. She was born to a healthy mother and a healthy father. At 36 and 5/7 weeks estimated gestational age, she was diagnosed with intrauterine growth retardation (IUGR) and oligohydramnios. She was born by normal spontaneous vaginal delivery at 40 and 1/7 gestational weeks. The birth weight was 2,280 g (−2.3 SD). Immediately after birth she was admitted to a neonatal intensive care unit (NICU) because of low birth weight, tachypnea, and suspected CdLS given her facial features. After several examinations and an increase in weight, she was discharged from the NICU. Her psychomotor development was severely delayed.

Though now 2 years old, she cannot sit without support or speak any words. Her voice is low-pitched. She had characteristic features of CdLS, including hirsutism, synophrys, arched eyebrows, long curly eyelashes, and a low frontal hairline. She had microcephaly, hypertelorism, a flat nasal bridge, a long philtrum, thin lips, a high arched palate, micrognathia, small hands and feet, single flexion crease on fifth finger, and micromelia. Other characteristic features are listed in Table I. (The family declined publication of a facial photograph.)

TABLE I. Comparison of Clinical Features in Our Case, Other Reported CdLS and del(1)(q25)

Feature	Present case	CdLS[a]	Typical CdLS[b]	Mild CdLS[b]	del1q23-25[c]
Psychomotor retardation	+		18/22	6/8	20/20
Growth retardation	+		19/20	7.5/8	17.5/19
Synophrys	+	99%	22/22	7/8	
Long eyelashes	+		22/22	5/8	
Hirsutism	+	78%	20/22	7/8	
Sparse hair or eyebrows	−				10/14
Hypertelorism	+		7/21	4/8	9/13
Broad and depressed nasal bridge	+	83%	17/22	4/8	5/10
Anteverted nostrils	−	88%	19/22	6/8	2/4
Prominent philtrum	+	94%	18/22	6/8	6/7
Small nose	+		14/22	3/8	
Thin lips	+	94%	22/22	6/8	6/7
Downturned angles of mouth	+	94%	20/22	7/8	8/9
High arched palate	+	86%	5/22	7/8	5/6
Low-set ears	−	70%	8/22	2/8	14/18
Micrognathia	+	84%	8/22	6/8	11/14
Short neck	+	66%			7/10
Microcephaly	+		15/19	7/8	17/18
Grossly malformed Upper limbs; oligodactyly	−	27%			
Micromelia	−				
Small hands and feet with short digits	+	93%	14/22	6/8	16/17
Proximally placed thumbs	+	72%	9/21	6/8	1/2
Clinodactly of fifth finger(s)	+[d]	74%	13/21	5/8	16/16
Small feet	+		13/21	5/8	2/2[e]
Low-pitched cry	+	74%	12/19	3/6	

[a]Three hundred ten cases of Jackson et al.
[b]Selicorni et al.
[c]Nine cases of Franco, ten cases of Pallotta, Hoeglund and Takano.
[d]With a single flexion crease of the fifth fingers.
[e]One case has short and broad toes, and the other talipes equinovarus.

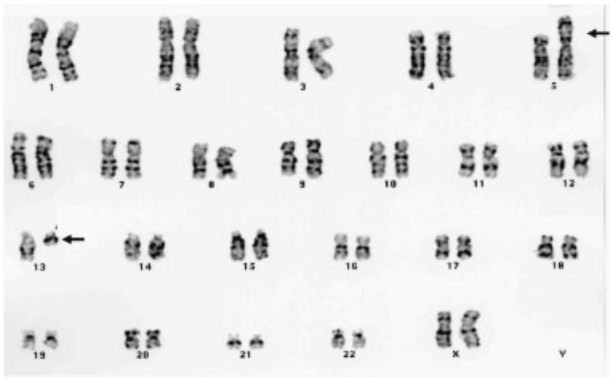


FIG. 1. G-banded chromosome from the proband. A translocation of 5p13 and 13q12 was detected. Arrows denote the translocated chromosomes.

Echocardiogram detected an atrial septal defect (ASD). She had moderate to severe bilateral hearing loss. A conventional cytogenetic examination showed a 46,XX,t(5;13)(p13.1;q12.1) de novo karyotype (Fig. 1). Karyotypes of her parents were normal.

Fluorescence In Situ Hybridization (FISH) Analysis

Metaphase chromosomes were prepared from normal male lymphocytes and from the patient's lymphoblastoid cell line (LCL) by standard methods. FISH analyses were performed as described previously [Ariyama et al., 1995], using a BAC located around the region of interest as probes. In order to confirm the translocation, painting probes for chromosomes 5 and 13 (Vysis, Chicago, IL) were also used.

Array-CGH Analysis

Since it was unclear whether only a disruption of *NIPBL* could explain all clinical manifestations of this patient including her atypical features (some of her facial features, clinodactyly and small hands) an array-CGH analysis was performed using our in-house MCG Whole Genome Array-4500 [Inazawa et al., 2004]. The MCG Whole Genome Array-4500 [Inazawa et al., 2004], which contains 4532 BAC/PAC clones covering the entire genome at intervals of approximately 0.7-Mb, was used for analysis of the present case and additional four cases of CdLS, three of whom had typical CdLS and the other an atypical mild (Table II). Hybridizations were carried out as described elsewhere [Sonoda et al., 2004; Hayashi et al., 2005] with a minor modification. Briefly, test and reference samples of genomic DNA from the patient's LCL and from peripheral blood mononuclear cells of a normal female volunteer were labeled with Cy3- and Cy5-dCTP (Amersham Biosciences, Tokyo, Japan), respectively, precipitated together with ethanol in the presence of Cot-1 DNA, re-dissolved in a hybridization mix (50% formamide, 10% dextran sulfate, 2x SSC, and 4% SDS, pH 7), and denatured at 75°C for 8 min. After 40 min of preincubation at 42°C, the mixture was applied to array slides, and incubated at 50°C for 10 min, 46°C for 10 min, and at 43°C for 60 hr in a hybridization machine, GeneTAC (Harvard Bioscience, Holliston, MA). The slides were then washed once in a solution of 2x SSC for 10 min at 50°C, 50% formamide, 2x SSC (pH 7.0) for 10 min at 50°C, and 1x SSC for 10 min at 42°C, respectively, and scanned with a GenePix 4000B (Axon Instruments, Foster City, CA). Acquired images were analyzed with GenePix Pro 6.0 imaging software (Axon Instruments). Fluorescence ratios were normalized so that the mean of the middle third of $\log_2$ ratios across the array was zero. Average ratios that deviated significantly (>2 SD) from 0 were considered abnormal.

RESULTS

FISH Analysis for the Breakpoint of der(5)t(5;13)(p13.1;q12.1)

The t(5;13)(p13;q12) translocation was confirmed using FISH with painting probes for chromosomes 5 and 13 (data not shown). All 20 of the lymphocytes analyzed showed the same aberration. In order to define the breakpoint precisely, a FISH analysis was performed using BACs at 5p13.1 (Fig. 2A). The signals specific for the BAC RP11-14I21 overlapping with the *NIPBL* gene were detected on both der(5) and der(13) (Fig. 2B), whereas signals for BAC RP11-758H16 proximal to RP11-14I21 were detected on both normal chromosome 5 and der(5). In addition, FISH signals for RP11-260B3 distal to RP11-14I21 appeared on both the normal chromosome 5 and der(5). The results indicate that the breakpoint at der(5) lies within *NIPBL*, and none of the visible deletions of genomic material around the breakpoint is involved in the translocation, at the molecular cytogenetic level.

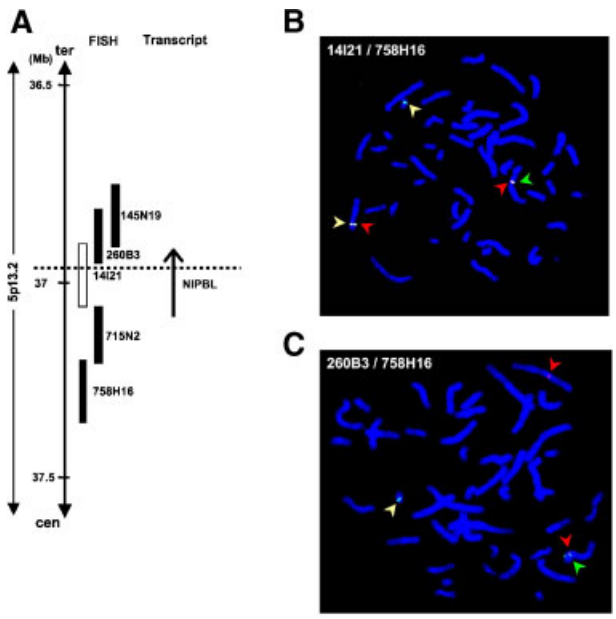
Array-CGH Analysis

Analysis of the ratio profiles of the array-CGH showed no aberration at 5p13.1 and 13q12.1. The data supported the FISH results described above, and indicate that her translocation observed is balanced. However, a decrease in the ratio of one BAC clone (RP11-162L13) at 1q25 was detected (Fig. 3A,B), suggesting that the chromosomal region spanning the BAC lost one allele of the genomic DNA sequence. To confirm this result, we carried out an additional array-CGH by inverting two fluorescents: the genomic DNA of the patient was labeled with Cy5 and that of a normal female was labeled with Cy3. The same result was obtained in this dye-swapping analysis (data not shown).

TABLE II. Clinical Features in Present Case and Other Four Cases of CdLS

Feature	CdLS1	CdLS2	CdLS3	CdLS4	CdLS5
Sex	F	M	M	F	F
Karyotype	46,XX,t(5;13)(p13.1;q12.1)	46,XY	46,XY	46,XX	46,XX
Array-CGH	del(1)(q25q31.1)[a]	NAD	NAD	NAD	NAD
NIPBL mutation	ND	exon43 7308delC	exon42 7216insCACA	negative	exon9 922C>T; R308X
Typical/atypical CdLS	typical	typical	typical	atypical	typical
Psychomotor retardation	+	+	+	+/−[b]	+
Growth retardation	+	+	+	+/−[b]	+
Synophrys	+	+	+	+	+
Long eyelashes	+	+	+	+	+
Hirsutism	+	+	+	+	+
Sparse hair or eyebrows	−	+	+	−	+
Hypertelorism	+	+	+	+	+
Broad and depressed nasal bridge	+	+	+	+	+
Anteverted nostrils	−	+	+	−	+
Prominent philtrum	+	+	+	+	+
Small nose	+	+	+	+	+
Thin lips	+	+	+	+	+
Downturned angles of mouth	+	+	+	+	+
High arched palate	+	+	+	+	+
Low-set ears	−	+	+	+	+
Micrognathia	+	+	+	+	+
Short neck	+	−	−	−	−
Microcephaly	+	+	+	+	+
Grossly malformed upper limbs; oligodactyly	−	−	−	−	+
Micromelia	−	+	+	−	+
Small hands and feet with short digits	+	+	+	+	+
Proximally placed thumbs	+	−	−	−	−
Clinodactly of 5th finger(s)	+[c]	−	−	+	+
Small feet	+	+	+	+	+
Low-pitched cry	+	+	+	+	+

ND, not determined; NAD, no possible pathogenic aberration was detected.
[a]For details see Results Section.
[b]Moderate psychomotor/growth retardation.
[c]With a single flexion crease of the fifth fingers.



FISH Analysis Around the Deleted Region at 1q25

In order to confirm the cryptic heterozygous loss at 1q25 and define its precise location, we performed FISH on the cells from the patient and her parents using a BAC (RP11-162L13), which is spotted on MCG Whole Genome Array-4500, and seven additional BACs (RP11-816G21, 591N8, 164P5, 104F23, 186D24, 809F11, and 136D14) (Fig. 4A,B). In the patient, six BACs (RP11-162L13, 164P5, 104F23, 186D24, and 809F11) showed a specific signal on one of homologous chromosomes 1 but not on the

FIG. 2. **A**: Scheme of the breakpoint at 5p13.2. The open vertical bar denotes the split BAC clone, RP11-14I21, the upward vertical arrow denotes the position of *NIPBL* which is a causative gene for CdLS, and filled vertical bars denote other BAC clones used in the FISH analysis. The dashed line indicates the breakpoint of the translocation. **B**,**C**: Representative results of FISH analysis. In (B), RP11-14I21 is labeled in green and RP11-758H16 is labeled in red. The green arrowhead denotes intact RP11-14I21, whereas the two yellow arrowheads denote split RP11-14I21. Red arrowheads denote RP11-758H16. In (C), RP11-260B3 is labeled in green and RP11-758H16 is labeled in red. Green arrowhead denotes RP11-260B3 on normal chromosome 5, whereas yellow arrowhead denotes one on derivative chromosome. Red arrowheads denote RP11-758H16. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

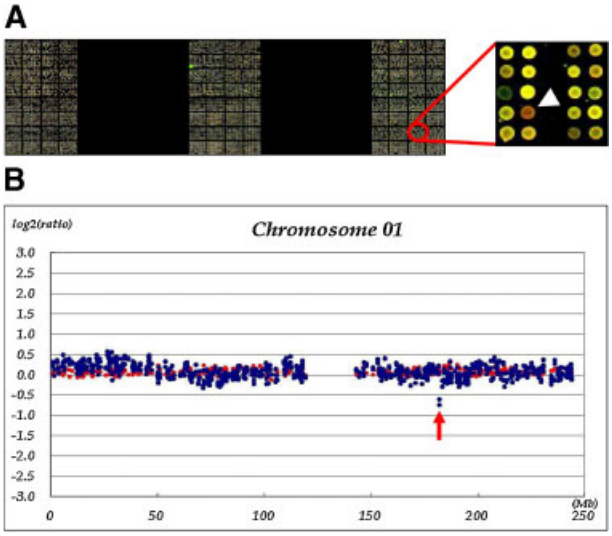


FIG. 3. The result of the array-CGH analysis using genomic DNA from the patient (labeled with Cy3) and a normal male (labeled with Cy5) as the test and control sample, respectively. **A**: Scanned image of the hybridized array (**left panel**) and partially magnified image (**right panel**). Each spot corresponds to a BAC clone. Yellow and red spots indicate clones with a normal copy number and reduced copy number in the test sample (white arrowhead), respectively. **B**: The copy number ratio ($\log_2$ ratio) of each spot (BAC clone) on chromosome 1. Cy5:Cy3 ratio of fluorescence intensity of each spot is normalized, and plotted as a $\log_2$ ratio. Each dot is ordered linearly such that the leftmost clone corresponds with the p-arm terminus and the rightmost clone corresponds with the q-arm terminus. The central gap corresponds with the centromere. Blue and red dots show the data for the present patient and the average for two different normal control DNAs, respectively. Red arrows denote the deleted region of 1q25. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

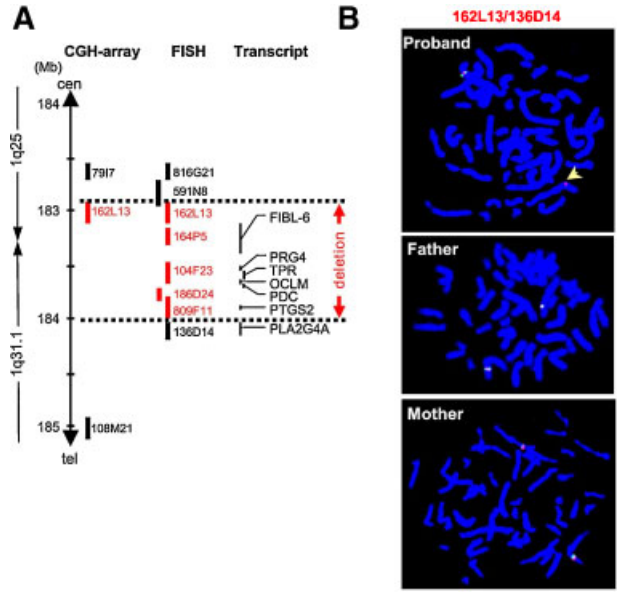


FIG. 4. **A**: Scheme of the interstitial deletion of 1q25–1q31.1. Thick vertical bars denote BAC clones. Red and black bars indicate deleted and retained clones, respectively. Clones drawn on the left side (RP11-79I7, RP11-162L13, and RP11-108M21) were used in the MCG Whole Genome Array-4500, while the others are additional clones used in the FISH analysis. Red closed arrows and two dashed lines indicate the deleted region confirmed by FISH. Thin vertical bars denote seven transcripts located around the deleted region. **B**: Representative results of FISH analyses using BAC clones on 1q25 (RP11-162L13, green) and 1q31.1 (RP11-136D14, red) on metaphase chromosomes of the patient (top), her father (middle), and her mother (bottom). One of two green signals indicating a clone in the deleted region has disappeared on the patient's chromosome (top; yellow arrowhead), whereas on her parents' chromosome, both green signals are detected (middle and bottom). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

other, although the specific signals for each of four BACs (RP11-79I7, 816G21, 591N8, and 136D14) were detected on both chromosomes 1. While in her parents, FISH signals specific for all eight BACs were detected on the long arms of homologous chromosomes 1 (Fig. 4B and data not shown). Taken together, the deletion at chromosome 1 detected by array-CGH is de novo, and is estimated to be approximately 1 Mb and occur at 1q25–q31.1 (182, 448, 509-183, 493, 303 bp; http://genome.ucsc.edu/, March 2006 assembly). It is described as arr cgh 1q25q31.1 (RP11-162L1 → RP11-809F11) × 1, according to ISCN 2005 [Shaffer and Tommerup, 2005].

Array-CGH Analyses for Other Cases of CdLS

Since an unanticipated genomic aberration was detected in this patient, we also analyzed four additional cases of CdLS by the MCG Whole Genome Array-4500 system. Two patients were males and the other two were females. Clinical features and the result of *NIPBL* mutation are listed in Table II. All cases were examined for *NIPBL* mutation, and three typical cases had a point mutation. However, no genomic (copy-number) aberrations were detected by array-CGH in any cases.

DISCUSSION

The molecular characterization of breakpoints involved in certain chromosomal translocations detected in patients with congenital disorders having the characteristic phenotype have occasionally allowed to identify causative genes for these diseases. Indeed, *NIPBL*, which encodes delangin, a homologue of the fungal Scc2-type sister chromatid cohesion and the Drosophila Nipped-B developmental regulator, was identified as a causative gene for CdLS from a patient with de novo t(5;13)(p13.1;q12.1) [Krantz et al., 2004; Tonkin et al., 2004]. Surprisingly, we found the same de novo t(5;13)(p13.1;q12.1) in the patient we have described. Fine mapping by FISH clearly showed that *NIPBL* was disrupted at the 5p13.1 breakpoint, resulting in the CdLS phenotype due to a haploinsufficiency of *NIPBL*, similar to the previous reports by Krantz et al. [2004] and Tonkin et al. [2004].

Mutations in *NIPBL* have been found in 27–56% of patients with CdLS [Borck et al., 2004; Gillis et al., 2004; Krantz et al., 2004; Tonkin et al., 2004; Bhuiyan et al., 2006; Miyake et al., 2005; Yan et al., 2006]. Gillis et al. [2004] extensively investigated a correlation

between genotype and phenotype including limb defects, growth, and development. The severity of limb defects had a similar trend, although it was not statistically significant. They also reported that a subset of individuals with mild phenotype of CdLS, despite having no mutation in *NIPBL*, might have a different genetic etiology from those with a mutation in *NIPBL*. Similarly, Yan et al. [2006] reported that mutation-positive patients were more severely affected than mutation-negative individuals with respect to some features, for example, birth weight or facial dysmorphism, but not others, limb defects, growth, and development. Bhuiyan et al. [2006] also reported that truncating mutations generally caused a more severe phenotype but the correlation was not absolute, and suggested that other factors were important to the phenotypic expression. These results suggest that *NIPBL* mutations tend to correlate with the severity of CdLS, although possible involvement of other genetic or environmental factors might modify the phenotype. More recently, indeed, Musio et al. [2006] reported a mutation of the *SMC1L1* gene at Xp12 in a case of familial CdLS with no mutation of *NIPBL*.

A number of chromosomal aberrations unrelated to loci for *NIPBL* and *SMC1L1* have been reported in individuals with CdLS [DeScipio et al., 2005]. Borck et al. [2004] reported that isochromosome 18p and a subtelomeric deletion of 1q were detected in two patients without a mutation in *NIPBL*. Notably, der(3)t(3;12)(p25.3;p13.3) with del(3)(p25) was detected in two half siblings with CdLS without an *NIPBL* mutation, suggesting genetic heterogeneity of CdLS [DeScipio et al., 2005]. These observations indicate that CdLS is a complex disorder, and certain chromosomal aberrations other than *NIPBL* mutations may also be causative for the CdLS phenotype, or be related to clinical features resembling CdLS. Thus, we carried out an array-CGH analysis to explore submicroscopic chromosomal aberrations in our CdLS patient who had some atypical characteristics. Consequently, a cryptic interstitial deletion at 1q25–q31.1 was detected.

The chromosomal deletions at 1q25 have been rarely reported [Franco et al., 1991; Takano et al., 1997; Pallotta et al., 2001; Hoglund et al., 2003]. Table I summarizes clinical features of 1q25 deletion and their frequency among our case, reported cases of typical and mild features of CdLS [Jackson et al., 1993; Selicorni et al., 1993], and cases of del(1)(q25). Interestingly, some of the clinical features in patients with del(1)(q25) overlap those in cases of mild CdLS. Hirsutism and synophrys are characteristic of CdLS, whereas minor anomalies of hands or feet, for example, small hands or clinodactyly of the 5th fingers, are not always observed in cases of CdLS but frequently observed in patients with del(1)(q25). Considering that mutations of *NIPBL* do not always correlate with limb defects as previously described, the features of our patients may have been affected by not only the disruption of *NIPBL* but also del(1)(q25). In our case, the deletion at 1q25 encompasses six genes (*FIBL-6*, *PRG4*, *TPR*, *OCLM*, *PDC*, and *PTGS2*) and some of them might be associated with her features. The *PRG4* gene is expressed in the cartilage [Ikegawa et al., 2000], and double-knockout mouse of *PRG4* revealed morphologic changes in the joints [Rhee et al., 2005]. Therefore, it is possible that haploinsufficiency of *PRG4* might be responsible for joint malformations such as clinodactyly in our case. Although the *PTGS2* has been reported to be relevant to prostaglandin synthesis, it is not clear whether heterozygous loss of *PTGS2* might be affected with any of her features.

We analyzed additional four cases of CdLS using array-CGH (Table II). Although one of them (CdLS4) showed mild CdLS without *NIPBL* mutation, no genomic aberration was detected in all cases.

In complex diseases like CdLS, even if the causative genes have been identified, other cryptic genomic aberrations are likely to affect the phenotype. High-throughput tools for the genome-wide scanning of cryptic chromosomal aberrations, including array-CGH, would facilitate the detection of unexpected submicroscopic chromosomal aberrations which may affect the complex phenotype in patients. In our case, indeed, the array-CGH was worth performing in order to detect genomic aberrations possibly affecting atypical features of CdLS.

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