Research Letter

Four Novel *NIPBL* Mutations in Japanese Patients With Cornelia de Lange Syndrome

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NIBPL mutations in de Lange Syndrome Patients

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To the Editor:

Cornelia de Lange syndrome (CdLS, OMIM #122470) is a multiple congenital anomaly syndrome characterized by dysmorphic facial features, hirsutism, severe growth and developmental delay, and malformed upper limbs [Jackson et al., 1993; Ireland et al., 1993]. The prevalence is estimated to be 1/10,000 [Opitz, 1985]. Recently, two independent groups proved that CdLS is caused by *NIPBL* mutations [Tonkin et al., 2004; Krantz et al., 2004]. *NIPBL* consists of 47 exons and encodes delangin, a 2,804 amino-acid protein, from exon 2 to 47.

We analyzed 15 Japanese sporadic patients (CdL 1-15) with typical CdLS features (Table I) and their parents after obtaining written informed consent. All protocols in this study were approved by the Committee for the Ethical Issues on Human Genome and Gene analysis, Nagasaki University. Clinical geneticists diagnosed these patients based on mental and growth retardation, and characteristic facial features. Genomic DNA was extracted using a standard protocol. Fourty-six coding exons (from exon 2 to 47) of *NIPBL* were amplified by PCR as described previously [Krantz et al., 2004] except for exons 4, 33, 37 and 41, of which primers were originally designed (available on request). Sequence analysis was performed as described previously [Kurotaki et al. 2003].

We identified three novel nonsense mutations and one missense mutation in

NIPBL among the 15 Japanese patients examined: 1885C>T (R629X) (CdL 4) and 1921G>T (E641X) (CdL 2) in exon 10, 3346G>T (E1116X) (CdL 15) in exon 12 and 5483G>A (R1828Q) (CdL 10) in exon 29. All the four mutations were not found in any of 97 normal Japanese controls or in the JSNP database (http://snp.ims.u-tokyo.ac.jp/). The altered amino acid (R1828Q) was *de novo* and located in the evolutionally conserved sequences at least in the human, rat, mouse and fly homologs, thus the change is likely to be pathological.

The C-terminal half ~1500 amino acids of delangin is well conserved among homologs of flies, worms, plants, and fungi, and is expected to be biologically important [Tonkin et al., 2004], though it was not found to contain any obvious functional domains by analysis using PROSITE (http://kr.expasy.org/cgi-bin/prosite/PSScan.cgi). Three protein truncation mutations at amino acid positions 629, 641 and 1116 and a missense mutation at amino acid position 1828 could lose or impair the C-terminal half function. The *Drosophila* homolog of NIPBL, Nipped-B, is involved in activating the *Ubx* and *Cut* homeobox genes. *Ubx* suppresses the limb formation by repressing *Dll* that requires for the distal limb development, and *Cut* mutations cause leg and wing abnormalities [Tonkin et al., 2004]. Thus, it is plausible that reduced expression of human *NIPBL* may lead to limb

anomalies in CdLS.

Interestingly, limb abnormalities (oligodactyly and ulner deficiency) were observed in 3 of our 4 patients with a mutation, but only one of 7 patients without any mutation whose clinical information was available did show some limb abnormality (oligodactyly), though Gillis et al. [2004] reported that severity of limb defects was not statistically different between mutation-positive and mutation-negative patients.

Additionally, three single nucleotide polymorphisms (SNPs), 1151A>G (N384S) in exon 9, 2021A>G (N674S) in exon 10 and 5874T>C (S1958S) in exon 33, were identified, as they were found among normal controls and the second substitution (2021A>G) was previously reported as a SNP [Gillis et al., 2004]. Allele frequencies of the three SNPs in normal Japanese controls are 3.2 % (6/186), 13.0 % (25/192) and 64.5 % (129/200), respectively.

To exclude a submicroscopic deletion around *NIPBL* and its franking regions, fluorescence *in situ* hybridization (FISH) analysis was performed in 10 of 15 cases on their metaphase chromosomes using 2 BAC clones covering the *NIPBL* gene (Table I), RP11-14I21 and RP11-7M4, selected from the UCSC genome browser, 2003 July version (http://genome.ucsc.edu/cgi-bin/hgGateway). FISH and subsequent photomicroscopy were performed as described previously [Miyake et al., 2004].

However none of them showed any deletion.

We also investigated core promoter regions in 11 affected individuals not having detectable point mutations in the coding regions. Two core promoter regions were identified, raging -800 to -500bp (CPR-A) and -400 to +200bp (CPR-B) from the beginning of *NIPBL* cDNA (NM_015384.3) using four different promoter prediction programs: neural network promoter prediction program (http://www.fruitfly.org/seq_tools/promoter.html), human core-promoter finder (http://rulai.cshl.org/tools/genefinder/CPROMOTER/human.htm), promoter 2.0 prediction server (http://www.cbs.dtu.dk/services/promoter/), bioinformatics & molecular analysis section (http://bimas.dcrt.nih.gov/molbio/proscan/). No nucleotide changes were detected among the 11 patients in the two core promoter regions except for a part of CPR-B sequence (-60~+60) which was hardly determined due to high GC ratio (75.83 %), suggesting that promoter mutations in *NIBPL* is less likely.

In conclusion, we identified four novel *NIPBL* mutations and three SNPs. It is important to describe a full spectrum of phenotype in more patients with positive mutations and establish comprehensive diagnostic criteria.

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REFERENCES

Gillis LA, McCallum J, Kaur M, DeScipio C, Yaeger D, Mariani A, Kline AD, Li HH, Devoto M, Jackson LG, Krantz ID. 2004. NIPBL Mutational Analysis in 120 Individuals with Cornelia de Lange Syndrome and Evaluation of Genotype-Phenotype Correlations. Am J Hum Genet 75(4):610-623

Ireland M, Donnai D, Burn J. 1993. Brachmann-de Lange syndrome. Delineation of the clinical phenotype. Am J Med Genet 47(7):959-964.

Jackson L, Kline AD, Barr MA, Koch S. 1993. de Lange syndrome: a clinical review of 310 individuals. Am J Med Genet 47(7):940-946.

Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, Nowaczyk MJ, Toriello H, Bamshad MJ, Carey JC, Rappaport E, Kawauchi S, Lander AD, Calof AL, Li HH, Devoto M, Jackson LG. 2004. Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B. Nat Genet 36(6):631-635.

Kurotaki N, Harada N, Shimokawa O, Miyake N, Kawame H, Uetake K, Makita Y, Kondoh T, Ogata T, Hasegawa T, Nagai T, Ozaki T, Touyama M, Shenhav R, Ohashi H, Mende L, Shiihara T, Ohtsu S, Kato Z, Okamoto N, Nishimoto J, Lev

D, Miyoshi Y, Ishikiriyama S, Sonoda T, Sakazume S, Fukushima Y, Kurosawa K, Cheng FJ, Yoshiura K, Ohta T, Kishino T, Niikawa N, Matsumoto N. 2003. Fifty microdeletions among 112 cases of Sotos syndrome: low copy repeats possibly mediate the common deletion. Hum Mutat 22(5):378-387.

Miyake N, Tonoki H, Gallego M, Harada N, Shimokawa O, Yoshiura KI, Ohta T, Kishino T, Niikawa N, Matsumoto N. 2004. Phenotype-genotype correlation in two patients with 12q proximal deletion. J Hum Genet.49(5):282-284

Opitz JM. 1985. The Brachmann-de Lange syndrome. Am J Med Genet 22(1):89-102.

Tonkin ET, Wang TJ, Lisgo S, Bamshad MJ, Strachan T. 2004. NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. Nat Genet 36(6):636-641.