Mutation Analysis in Chinese Patients with Cornelia de Lange Syndrome

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Aims: Cornelia de Lange syndrome (CdLS) is a dominant multisystem developmental disorder and related to mutations of the *NIPBL*, *SMC1A*, and *SMC3* genes. So far, there has been no report of a mutation analysis in Chinese patients with CdLS, while 12 cases have been clinically described. In the present study, we tried to search for pathogenic mutations of the *NIPBL*, *SMC1A*, and *SMC3* genes in four patients with CdLS from four unrelated Chinese families. **Results:** The mutational analysis of the *NIPBL*, *SMC1A*, and *SMC3* genes by direct sequencing revealed a heterozygous splice-site mutation c.4321G>T(p.V1441L) at exon 20 of *NIPBL* in proband 2 and a novel heterozygous splice-site mutation c.6589+5G>C at intron 38 of *NIPBL* in proband 3, which was showed by reverse transcription polymerase chain reaction to generate both the full-length and an alternatively spliced transcript with an exon 38 deletion. **Conclusions:** This is the first report of the mutation analysis of *NIPBL* in China and our findings both expand the mutation spectrum of *NIPBL* and provide data for further understanding of the diverse and variable effects of *NIPBL* mutations.

Introduction

CORNELIA DE LANGE SYNDROME (CdLS) (MIM # 122470, # 300590 and # 610759) is an autosomal dominant and genetically heterogeneous disorder characterized by typical facial features, developmental and growth retardation, hirsutism, limb anomalies, and multiple organ involvement. The estimated prevalence of CdLS is between 1:10,000 and 1:13,000 live births, and most cases appear to be sporadic. CdLS was initially described by Vrolik (1849), and de Lange (1933) classified the clinical findings as a diagnostic entity. Three genes, *NIPBL* (5p13.1, MIM # 608667), *SMC1A* (Xp11.2, MIM # 300040), and *SMC3* (10q25, MIM # 606062) (Krantz *et al.*, 2004; Tonkin *et al.*, 2004; Musio *et al.*, 2006; Deardorff *et al.*, 2007), have been found to cause CdLS. Combined, heterozygous mutations in *NIPBL*, *SMC1A*, and *SMC3* contributed to ~65% of the CdLS patients with a confident clinical diagnosis (Deardorff *et al.*, 2007; Oliveira *et al.*, 2010) among various ethnicities, including Caucasians, Africans, and Asians.

Since CdLS was first reported in China (Qian and Zhou, 1982), 12 cases have been clinically described. However, there have been no reports of the mutation analysis for any genes associated with CdLS in Chinese patients. In the present study, we tried to search for pathogenic mutations of the *NIPBL*, *SMC1A*, and *SMC3* genes in Chinese patients with CdLS.

Materials and Methods

Patients

Four patients with CdLS-like phenotypes from four Han Chinese families (Fig. 1A) were referred to us for genetic diagnosis and counseling in 2010. Medical data and photographs were obtained routinely with particular attention being paid to the presence/absence of complications known to be related to CdLS. All patients presented with short stature and typical facial features in addition to other characteristic manifestations of CdLS (Fig. 1B and Table 1). Examination protocols were approved by the university ethics committees. Informed consent was obtained from all patients and their parents.

Mutation analysis by direct sequencing

Genomic DNAs were extracted from peripheral blood using standard techniques. All exons and flanking introns sequences of the *NIPBL*, *SMC1A*, and *SMC3* genes were amplified by polymerase chain reaction (PCR). PCR products were confirmed by polyacrylamide gel electrophoresis (PAGE), and directly sequenced with the ABI PRISM BigDye kit on an ABI3130 DNA sequencer. The same experiment was carried out on the patients' parents and 100 unrelated normal controls to determine whether the detected nucleotide changes were causative mutations or genetic polymorphisms.

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FIG. 1. (A) Pedigrees of present four families. (B) Facial features of present 4 patients.

Reverse transcription-PCR analysis of splice-site mutation

Total RNA was extracted from peripheral blood using the Trizol Plus RNA Purification Kit (Invitrogen) and cDNA was synthesized using the oligo (dT) primer and the SuperScript First-Strand Synthesis System (Invitrogen). Primers were designed spanning the sequences of the exon 38 and part of the exon 37/39 of the *NIPBL* cDNA (NM_015384). The forward primer was 5'-TCAACACCAAGAGGACCCAA-3', and the reverse primer was 5'-GTCTCTATCTGCCTGCTGCATA-3'. Reverse transcription (RT)-PCR products were separated by electrophoresis in 1.2% agarose gel, recycled, and purified with the QIA quick Gel Extraction Kit (Qiagen), and then sequenced respectively.

Genome-wide copy number variation analysis

The genome-wide copy number variation (CNV) analysis was performed using the Illumina Human 660W-Quad BeadChip (consisting of 657,366 SNP markers) in patient 1 and 4. The total of DNA input was 200 ng for each sample.

DNA amplification, tagging, and hybridization were performed according to the manufacturer's protocol. The array slides were scanned on an iScan Reader (Illumina). Data analyses were performed using the GenomeStudio version (2010.1). UCSC-built Hg18 (Human Mar. 2006 (NCBI36/hg18) Assembly) was used to analyze the data. CNVs were checked in DGV databases (<http://projects.tcag.ca/variation/>).

Results

Dideoxynucleotide sequencing showed a heterozygous c.4321G>T variant at the first base of exon 20 that affected the 3' splice acceptor site of intron 19 of *NIPBL* in patient 2 (Fig. 2A), which is previously described (Pie *et al.*, 2010), and a heterozygous c.6589+5G>C mutation in the 5' splice donor site of intron 38 of *NIPBL* in the patient 3 (Fig. 2B), which has not been reported previously by querying the Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk/ac/gene.php?gene=NIPBL) and NIPBL-LOVD database (www.lovd.nl/NIPBL). No mutation of *NIPBL*, *SMC1A*, and *SMC3* and pathogenic or unknown CNV was identified in patients 1

TABLE 1. CLINICAL CHARACTERISTICS OF FOUR PATIENTS IN THE PRESENT STUDY

<i>Patient</i>	<i>Gender</i>	<i>Age at test</i>	<i>Birth weight</i>	<i>Facial features</i>	<i>Limb abnormalities</i>	<i>Other abnormalities</i>	<i>Mutation</i>
1	M	5 years 6 months	2600 g	Synophrys; long eyelashes, depressed nasal root with upturned nasal tip and anteverted nares; long philtrum, thin upper lip; small widely spaced teeth; brachymicrocephaly; ptosis	Short terminal phalanges of the fifth fingers	Neonatal behavioral neurological development test at birth indicated neurobehavioral development abnormalities; hirsutism	NA
2	F	2 years 8 months	2750 g	Synophrys; long eyelashes, depressed nasal root with upturned nasal tip and anteverted nares; long philtrum, thin upper lip; brachymicrocephaly	Small hands with short digits	E2: 46.89 pM, T: 0.069 nM; hirsutism	NIPBL c.4321G>T
3	F	19 months	2500 g	Synophrys; long eyelashes; depressed nasal root with upturned nasal tip and anteverted nares; long philtrum; thin upper lip; brachymicrocephaly;	Short fifth fingers with two knuckles; single transverse palmar crease (right hand)	Metabolic screening at age 30 days indicated a reduced level of acyl carnitines; acleistocardia; hirsutism	NIPBL c.6589+5G>C
4	F	50 days	3000 g	Synophrys; long eyelashes; depressed nasal root with an upturned nasal tip and anteverted nares; long philtrum, thin upper lip; brachymicrocephaly; low-set ears;	Small hands with single transverse palmar crease	Cleft palate; acleistocardia; hirsutism	NA

M, male; F, female; NA, not available.

FIG. 2. (A) Sequencing showing the c.4321G>T heterozygous mutation in exon 20 of *NIPBL* in patient 2. Arrow denotes the mutation site. (B) Sequencing showing the c.6589+5G>C heterozygous mutation in intron 38 of *NIPBL* in patient 3. Arrow denotes the mutation site. Exon sequences are in uppercase letters, and intron sequences in lowercase letters (A, B). (C) Reverse transcription-polymerase chain reaction (RT-PCR) products of the *NIPBL* cDNA showing two bands of 397 and 307 bp in the patient 3 (lane B) and one 397 bp band in the normal control (lane A). (D) RT-PCR products sequencing showing that the c.6589+5G>C mutation caused a splicing error in the exon 38 of *NIPBL* and generated both full-length and an alternatively spliced transcript with an exon 38 deletion. Arrows denote the splice junctions of exon 37/38 and exon 37/39, respectively, in the full-length and the alternatively spliced transcript.

and 4. The heterozygous c.4321G>T and c.6589+5G>C mutations of *NIPBL* were not found in all parents of the patient 2 and 3, as well as 100 unrelated healthy controls.

RT-PCR of the *NIPBL* cDNA showed that two bands of 397 and 307 bp were amplified, respectively, in patient 3, while only one 397 bp band was amplified in the normal control (Fig. 2C). The RT-PCR products sequencing showed that the heterozygous c.6589+5G>C mutation caused loss of function of a splice donor site of intron 38, resulting in a full-length and an alternatively spliced transcript with an exon 38 deletion (Fig. 2D).

Discussion

NIPBL encodes for delangin, the human orthologue of the *Drosophila melanogaster* Nipped-B, which is a key protein responsible for the correct development of many organs in the growing embryo. *NIPBL* contains 47 exons and is predicted to generate isoforms of 2804 or 2697 amino acids. The observed expression pattern of *NIPBL* is consistent with the CdLS phenotype (Krantz *et al.*, 2004; Tonkin *et al.*, 2004). Nipped-B is thought to regulate the effect of cohesion on transcription by dynamic control of cohesin binding, or of subunit interactions (Dorsett and Krantz, 2009). Cohesin is responsible for sister chromatid cohesion, ensuring correct chromosome segregation during the mitotic and meiotic cell cycles. Beyond this role, cohesin and regulatory cohesin genes seem to play a role

in preserving genome stability, DNA repair, and gene transcription regulation (Liu and Krantz, 2009). There is increasing evidence that many of the developmental deficits in CdLS likely result from changes in gene expression (Dorsett and Krantz, 2009).

According to the Locus Specific Databases (LSDB) 8, *NIPBL* mutations have since been described in upto 56% of CdLS patients. To date, the *NIPBL*-LOVD database contains 233 unique mutations reported in 305 patients among various ethnicities, including Caucasians, Africans, and Asians, involving most exons.

The genotype-phenotype correlation of CdLS is still not clear, as in previous reports, splice-site mutations appeared to cause variable phenotype (Oliveira *et al.*, 2010; Pie *et al.*, 2010). Selicorni *et al.* (2007) reported that most of the splice-site and all missense mutations were found in the patients with a low-medium clinical score. In the present study, we detected two *NIPBL* splice-site mutations that caused mild phenotype in patient 2 and patient 3 (Table 1). The splice-site of intron 19 mutation c.4321G>T in patient 2 was previously reported to cause the deletion of exon 20 and mild symptoms (Pie *et al.*, 2010). The novel splice-site of intron 38 mutation c.6589+5G>C in patient 3 induced skipping of exon 38 (91 bp) that caused a premature stop codon at the 28th base of exon 39. Based on the RT-PCR result showing that the 306 bp product band is slightly lighter than the 397 bp one (Fig. 2C), even though the resulting mRNA had partially been degraded by

nonsense-mediated decay, most of them could be translated into an alternative truncated protein while retaining partial function of the NIPBL protein and leading to a mild CdLS phenotype. Splice-site mutation in the same residue (c.6589+5G>A) was reported previously, but the patient's phenotype was not supplied by the author (Oliveira *et al.*, 2010).

Pie *et al.* (2010) found that CdLS patients with mutations in *SMC1A* had a higher incidence of high palate anomalies, but no mutation in *SMC1A* was identified in our patient 4 with cleft palate. It is possible that mutations affecting the remaining structural components of the cohesin complex (Rad21 or Stag2) or mutations in regulatory sequences for *NIPBL* might occur in some of the patients without detected mutations in target sequences of *NIPBL*, *SMC1A*, or *SMC3* (Liu and Baynam, 2010).

The role of *NIPBL* and cohesin in the etiology of the CdLS are not fully understood so far. In the present study, we identified two causative mutations of *NIPBL*, including a novel splice-site mutation, and offered a reliable diagnosis and genetic counseling in two of the four Chinese patients with CdLS. This is the first report of mutation analysis of *NIPBL* in China and our findings both expand the mutation spectrum of *NIPBL* and provide data for further understanding of the diverse and variable effects of *NIPBL* mutations.

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Author Disclosure Statement

No competing financial interests exist.

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