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Case Report

Two novel *NIPBL* mutations in three Chinese neonates with Cornelia de Lange syndrome identified by disease-associated genome panel

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Abstract: Cornelia de Lange syndrome (CdLS) is a rare dominantly inherited multisystem developmental disorder characterized by clinical and genetic heterogeneity. Five genes, *NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21*, have been found to cause CdLS. The genetic diagnosis of patients with clinically suspected CdLS is usually performed using gene by gene Sanger sequencing, but it has low diagnosis rate and is time-consuming and labor intensive. Here, we designed the disease-associated genome (DAG) panel, including about 3994 known Mendelian disease genes, and performed molecular diagnoses for 3 unrelated Chinese neonates with clinically suspected CdLS. The sequencing data revealed a previously reported frameshift mutation c.7438_7439delAG (p.K2480fsX3) and 2 novel mutations, including a heterozygous nonsense mutation c.1904C>G (p.S635X) and a missense mutation c.6647A>G (p.Y2216C) in the *NIPBL* gene. These results extend the mutation spectrum of the *NIPBL* gene. We also showed that DAG panel sequencing would be an effective method for genetic diagnosis of patients with CdLS.

Keywords: Cornelia de Lange syndrome, disease-associated genome panel, *NIPBL*, mutation

Introduction

Cornelia de Lange syndrome (CdLS; MIM# 122470, 300590, 610759, 300882, and 614701) is a rare multisystem developmental disorder characterized by clinical and genetic heterogeneity [1]. The main clinical manifestations are typical facial features, intrauterine and postnatal growth retardation, microcephaly, shortening of the limbs, psychomotor delay, and intellectual disability [2, 3]. It is difficult to make an accurate diagnosis only according to clinical diagnostic criteria, especially for the neonates those whose phenotypes have not been yet observed or are less typical [1]. Therefore, a genetic diagnosis of patients with clinically suspected CdLS is needed to improve diagnostic efficiency.

CdLS is a genetically heterogeneous disorder due to mutations in the 5 cohesion complex genes (*NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21*) and chromosomal abnormalities, which is a great challenge for clinical molecular diagnosis of Cdls [4, 5]. The genetic diagnosis of patients with clinically suspected CdLS is usually performed using gene by gene Sanger sequencing, but the classical Sanger sequencing for all genes associated with CdLS and CdLS-like phenotypes is time-consuming and labor intensive because a significant number of patients with CdLS negative for known CdLS genes have somatic mosaicism [6-9]. Next-generation sequencing (NGS) is the newest, most efficient technology for clinical diagnostics and has been used for molecular diagnosis of some Mendelian diseases [10, 11]. One approach of NGS, whole-exome sequencing (WES), has been used for the molecular diagnosis of CdLS in several studies [4, 5, 12, 13]. But WES is costly and reveals numerous variants that can be a challenge for the interpretation of sequence variants. Target NGS can detect the functional regions of the interested genes for a specific disease or diagnostic category that are

Table 1. Summary of clinical phenotype and *NIPBL* mutations

	Patient 1	Patient 2	Patient 3
Gender	Girl	Boy	Girl
Gestational age (week)	37+2	36+1	38+6
Birth weight (gram)	2150	1800	2690
Head circumference at test (cm)	29	28	31
Height at test (cm)	45	41	44
Prenatal history			
Intrauterine growth retardation	+	+	-
Intrauterine distress	+	-	+
Microcephaly	+	+	+
Facial anomaly			
Synophrys	+	+	+
Long eyelashes	+	+	+
Thin upper lips	-	-	+
Depressed nasal bridge	-	+	-
Anteverted nares	-	+	-
Downturned corners of the mouth	-	+	-
Micrognathia	-	+	-
Limb abnormalities	+	+	+
Single transverse palmar crease	+	+	+
Hypotonia	+	-	-
Hirsutism	+	+	-
Mutation (*NIPBL*)	c.7438_7439delAG	c.1904C>G	c.6647A>G

amplified. For CdLS, however, there is no commercial target NGS panel. Therefore, we focused on the part of whole exome genome and designed the disease-associated genome (DAG) panel [14] panel including about 3994 known Mendelian disease genes and 24 targeted NGS panels based on clinical themes that are clinically interpretable.

Herein, we searched for pathogenic mutations in 3 Chinese neonates with a suspected clinical diagnosis of CdLS by DAG panel.

Materials and methods

Patients

Three neonates with clinically suspected CdLS were enrolled from the neonatal intensive care unit at Bayi Children's Hospital Affiliated to PLA Army General Hospital (Beijing, China). Three neonates came from three Han Chinese families. They all presented with microcephaly and typical facial features in addition to other characteristic manifestations of CdLS (**Table 1**). This study was performed with the approval of the Ethical Committee of PLA Army General Hospital and was conducted according to the tenets of the Declaration of Helsinki. Written consent was obtained from all the patients' guardians.

DAG panel sequencing

We designed a comprehensive catalog including 3994 Mendelian disease genes involving 24 disease panels (Table S1) using data from 2 parts: (1) 3595 genes in the Online Mendelian Inheritance in Man (OMIM) (December 20, 2015); (2) 399 genes the Human Gene Mutation Database (December 20, 2015).

We conducted DAG panel sequencing of the 3 neonates' DNA to identify the causal gene. The library was prepared using 3 μg DNA using the Agilent SureSelect Library Prep Kit. The final library size was 300 to 400 bp, including the adapter sequences. The sample was then tagged by PCR with different index sequences. The enriched library of the proband was sequenced on the Illumina HiSeq 2500 platform (Illumina) for paired-end reads of 100 bp in Precisionmdx.com (Beijing, China, http://www.precisionmdx.com/).

Sanger sequencing validation

The primers of candidate mutation regions were designed by Primer3 online (http://primer3.ut.ee/). The PCR products were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed by an ABI 3730 Genetic Analyzer (Applied Biosystems).

Results

Three variants in the *NIPBL* gene were detected in 3 patients by comprehensive gene panels

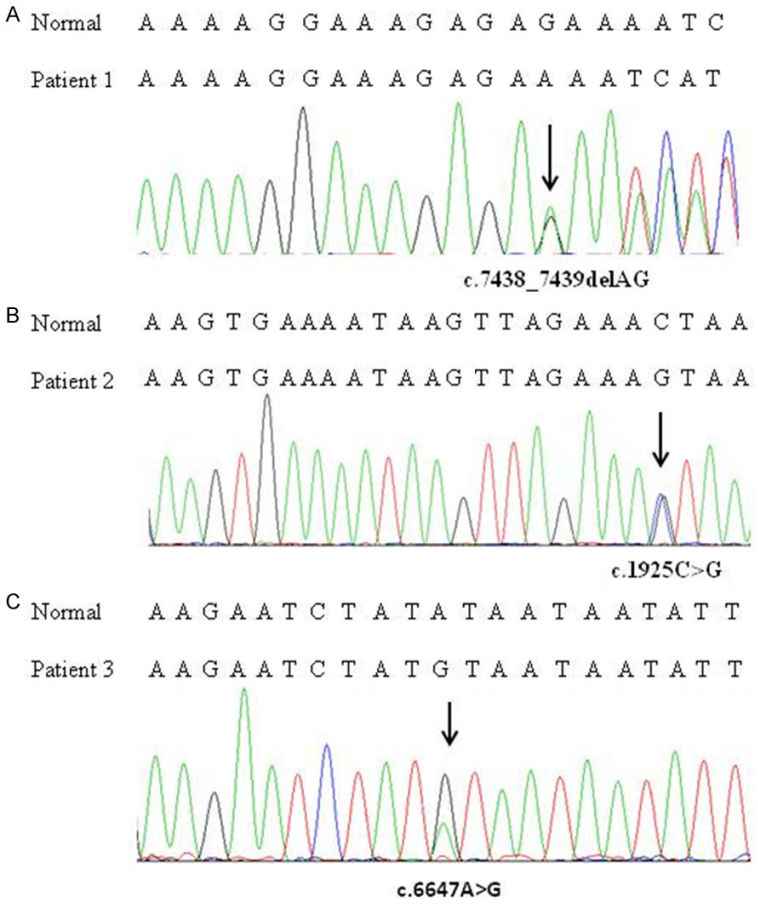

Figure 1. Sanger sequencing of *NIPBL* mutation analysis for patients 1-3. The arrow denotes the mutation site. A. Sequencing showing the c.7438_7439delAG heterozygous mutation in exon 44 of *NIPBL* in patient 1. B. Sequencing showing the c.1904C>G heterozygous mutation in exon 10 of *NIPBL* in patient 2. C. Sequencing showing the c.6647A>G heterozygous mutation in exon 39 of *NIPBL* in patient 3.

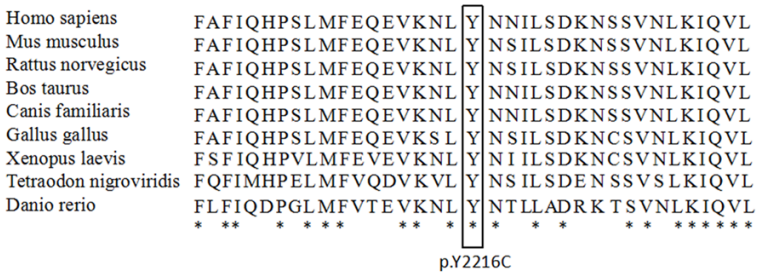

Figure 2. Conservation of the amino acid changed by the c.6647A>G (p.Y2216C) across different species.

and Sanger sequencing validation. Patient 1 was revealed to be a heterozygous pathogenic frameshift mutation, c.7438_7439delAG (p.K2480fsX3) in exon 44 of the *NIPBL* gene (**Figure 1A**), which was previously reported [15]. In patient 2, 1 novel heterozygous variant, c.1904C>G, was identified in exon 10 of the *NIPBL* gene. The c.1904C>G mutation resulted in a truncated treacle protein with a premature termination codon at the 635th amino acid (p.S635X) (**Figure 1B**). In patient 3, 1 novel heterozygous variant, c.6647A>G, was detected in exon 39 of the *NIPBL* gene, resulting in the replacement of tyrosine by cysteine at codon 2216 (p.Y2216C) (**Figure 1C**). The p.Y2216C mutation is conserved in all species examined (**Figure 2**) and predicted to be likely pathogenic in silico. None of these mutations were detected in 100 healthy control samples and were confirmed by Sanger sequencing.

Discussion

NIPBL is the human homolog of the *Drosophila* Nipped-B gene and encodes delangin, which is an essential protein for the correct development of many organs in the growing embryo [16]. It is located at 5p13 and is composed of 47 exons that are predicted to generate isoforms of 2804 or 2697 amino acids. According to the some reports, *NIPBL* mutations have been identified in about 60% of patients with CdLS [2, 15, 17, 18]. To date, the 278 mutations in 378 patients of various ethnicities have been reported in the NIPBL-LOVD database. The types of mutations detected in the *NIPBL* gene include frameshift, nonsense, missense, splicing mutations, and intragenic deletions [19]. In our study, 3 different *NIPBL* variants were detected in 3 neonates with CdLS. In patients 1 and 2, the c.7438_7439delAG (p.K2480fsX3) and c.1904C>G (p.S635X) mutations were identified, respectively, of which c.7438_7439delAG

had been previously reported [15]. These 2 mutations belong to loss of function (LOF) variants that include stop gain, splicing, and frameshift variants and lead to produce a prematurely truncated protein resulting in haploinsufficiency [18]. In patient 3, 1 novel c.6647A>G missense variant was detected. The c.6647A>G (p.Y2216C) variation was found to be likely pathogenic using SIFT, PolyPhen, and Mutation Taster prediction. The p.Y2216 amino acid is highly conserved throughout evolution and lies around the HEAT4 repeat (2227-2267) of the HEAT domain. HEAT repeats affect NIPBL's ability to interact with other proteins that are found in condensins, cohesins, and other complexes with chromosome-related functions. Many reported missense mutations show a clustering in and around the 5 HEAT repeats that have been identified in *NIPBL* [15, 20]. All these factors suggest that the c.6647A>G mutation might be a pathogenic change.

In recent years, the *NIPBL* genotype-phenotype correlation of CdLS has been studied in some reports [17, 18, 21, 22]; however, the results have been inconsistent. Some reports show that LOF mutations including nonsense, splicing-site, and frameshift mutations leading to a nonfunctional NIPBL are associated with a more severe phenotype characterized by typical facial features, severe growth and developmental retardation, and limb defects [18]. Patients with missense mutations show a milder phenotype including less growth and developmental delays and no limb abnormalities [18]. In contrast, Zhong et al. showed that two *NIPBL* splicing-site mutations caused mild a phenotype in 2 Chinese patients [17]. In the present study, patient 1, who carried a frameshift mutation, had a milder phenotype compared to patient 2, who carried a nonsense mutation. Patients 1 and 2 had intrauterine growth retardation and limb abnormalities, but patient 2 had more facial features such as a depressed nasal bridge, anteverted nares, downturned corners of the mouth, and micrognathia. Whether the more severe phenotype in patient 2 with the nonsense mutation p.S635X is caused by the position of the mutation should be further studied. In contrast to patients 1 and 2, patient 3, who carried a missense mutation, had a milder phenotype such as birth weight > 2500 g without intrauterine growth retardation and hirsutism. However, patient 3 had limb abnormalities, which was inconsistent with the report from Mannini et al. [18]. In addition, all patients are neonates whose phenotypes have not yet been observed, such as sitting position, walking independently, and speaking first words; hence, follow-up studies and additional cases are needed to understand the genotype-phenotype correlation in CdLS.

In conclusion, we performed DAG panel sequencing, an approach of NGS, and successfully detected 2 novel mutations and 1 already reported mutation in *NIPBL* for 3 Chinese neonates with clinically suspected CdLS. To the best of our knowledge, this is the first study that investigated the application of the DAG panel in the molecular diagnosis of CdLS in Chinese. Our results indicate that DAG has advantages in expenditure of time and money of each sample, coverage of targeted genes, and the ability to interpret the variants over the whole exome or genome.

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Disclosure of conflict of interest

None.

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Table S1. DAG panel involving 24 disease panels

NO.	Genes associated with diseases	Number of genes	NO.	Genes associated with diseases	Number of genes
1	Respiratory diseases	62	13	Amyotrophic Lateral Sclerosis	26
2	Craniofacial anomalies	113	14	Parkinson's disease and dysmyotonia	168
3	Skeletal anomalies	237	15	Cognitive disorder	113
4	Deaf	189	16	Hereditary ataxia and spastic paralysis	315
5	Ophthalmic diseases	258	17	Peripheral neuropathy	444
6	Diseases of the blood and immune system	535	18	Eukodystrophy	152
7	Pathoglycemia	51	19	Syndroms	816
8	Endocrine disease	52	20	Mental retardation	498
9	Gonadal development and function anomalies	179	21	Epilepsy	443
10	Congenital heart disease and Cardiomyopathy	231	22	Mitochondrial disease	618
11	Renal diseases	376	23	Inherited metabolic disorders	364
12	Hepatopathy	379	24	Genes associated with metabolic pathway	850