Mutation spectrum of *NIPBL* gene in Korean patients with Cornelia de Lange Syndrome

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Mutation spectrum of *NIPBL* gene in Korean patients with Cornelia de Lange Syndrome

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ABSTRACT

Mutation spectrum of *NIPBL* gene in Korean patients with Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome (CdLS, also called Brachmann de Lange Syndrome; OMIM 122470) is characterized by multiple malformations, severe growth, mental retardation, and distinctive facial features. Patients with CdLS show clinically variable phenotypes that range from mild to severe. The prevalence of CdLS is estimated to be about 1 in 10,000 ~ 30,000. Although CdLS is inherited as an autosomal dominant or X-linked manner, most cases appear to be sporadic. Currently, heterozygous mutations in the cohesin regulator *NIPBL*, or the cohesin structural components *SMC1A* (formerly *SMC1L1*) and *SMC3* have been identified as a cause of CdLS. *NIPBL* gene mutation has been identified in approximately 20 to 50% of CdLS cases, and 5% of patients show

mutations in the *SMC1A* gene. In this study, the mutation spectrum of *NIPBL* and *SMC1A* genes in 13 Korean patients with CdLS was investigated. Five patients (38%) showed mutations in *NIPBL* and one patient (9%) carried a mutation in *SMC1A* from the 13 unrelated Korean patients with CdLS. There were 5 novel heterozygous mutations including c.2108C>A (p.Pro703Gln), c.6425G>T (p.Arg2142Ile), c.6530T>C (p.Leu2177Ser), c.6679-6682delGTCA, c.7549delG identified in *NIPBL* and 1 novel heterozygous mutation, c.2327T>C (p.Val776Ala), in *SMC1A*. The rest of the 7 CdLS patients who showed negative results in mutation analysis for the *NIPBL* and *SMC1A* genes were screened by 4X180K array-based comparative genomic hybridization (array-CGH) to detect common chromosomal rearrangements that may suggest novel candidate CdLS genes. Only two patients showed DNA copy number alterations, including a deletion on chromosome 5q12.1 and a duplication on chromosome 16p11.2. The results of this study suggest the genetic heterogeneity of CdLS. Further analysis will be needed to identify novel candidate gene(s) responsible for CdLS.

Key Words : Cornelia de Lange Syndrome, *NIPBL*, *SMC1A*, array-based comparative genomic hybridization, genetic heterogeneity

Mutation spectrum of *NIPBL* gene in Korean patients with Cornelia de Lange Syndrome

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I. INTRODUCTION

Cornelia de Lange Syndrome (CdLS, also called Brachmann de Lange syndrome; OMIM 122470) is a rare, genetically heterogeneous disorder which is characterized by facial dysmorphisms, upper-extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, gastrointestinal abnormalities and hearing problems.[1-2] Patients with CdLS show clinically variable phenotypes that range from mild to severe.[1-2]

The prevalence of CdLS is difficult to estimate since individuals with milder phenotypes are unlikely to recognize their condition.[3] However, it is estimated to be around one in 10,000 to 30,000[4], and more than 99% of cases appear to be sporadic. This syndrome is inherited as an autosomal dominant or X-linked manner. There are recorded cases of unaffected parents who have affected children with CdLS. These cases are hypothesized to be the result of germline mosaicism, the recurrence risk of which is estimated to be 0.5% ~ 1.5%.[1]

Nipped B-Like (*NIPBL*) is a major gene currently known as a disease gene for CdLS.[5] The *NIPBL* gene, which is the human homolog of the *Drosophila* Nipped-B gene, is located on chromosome 5p13 and is made up of 2,804 amino acids (Fig 1). The function of the *NIPBL* gene product, called delangin, is involved in sister chromatid cohesion, in regulating long-range enhancer-promoter interactions, and in repairing of damaged DNA at G_2 phase.[6]

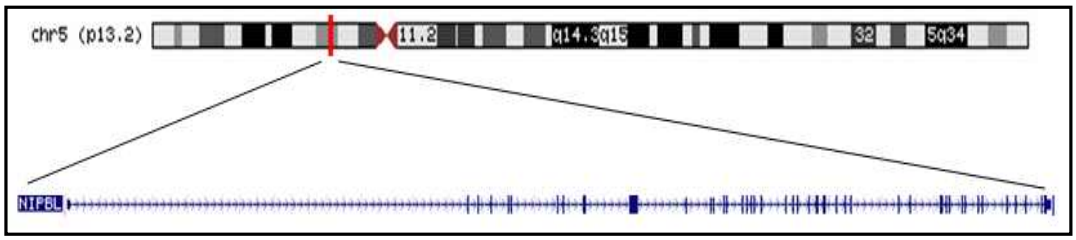

Figure 1. Genetic map of *NIPBL* gene. The *NIPBL* gene is located on chromosome 5p13 and is consisted of 47 exons.

Other disease genes causing CdLS are structural maintenance of chromosomes 1A (*SMC1A*) and structural maintenance of chromosomes 3 (*SMC3*) genes, both of which encode core components of the cohesin complex.[7] *SMC1A* gene, located on chromosome Xp11.22-p11.21 that escapes X inactivation in humans[8], contains 25 exons and is comprised of 123 amino acids. *SMC3* gene is located on chromosome 10q25 (Fig 2). The SMC1A and SMC3 subunits are rod-shaped molecules with globular ATPase domains at one end and dimerization domains at the other end, having intervening sequence forming antiparallel coiled-coil structure (Fig 3).[9] The two ATPase head domains interact with the non-SMC subunit (Rad21, Stromalins), creating a triangular structure that could trap sister chromatids.[9] The cohesion complex, which is consisted of SMC1A and SMC3, is involved in chromosome cohesion during the cell cycle and functions in DNA repair (similar to *NIPBL*[6]), and contributes to gene regulation in postmitotic cell (recently discovered).[10]

Over 100 causative *NIPBL* gene mutations have been reported. They include missense/nonsense (~42%), deletion/insertion (~40%), and splice-site mutations (~16%) (Table 1). The mutations types of the *SMC1A* gene consists missense/nonsense (~70%) and frameshift (~30%) mutations. One individual was found to have an in-frame 3 bp deletion (c.1464_1466del3) in the *SMC3* gene (Table 2). Mutations in *NIPBL* take up to 20 ~50% of clinically diagnosed CdLS cases.[11] *SMC1A* gene mutations contribute to 5% of CdLS cases and result in consistently milder phenotypes than patients with *NIPBL* gene mutations.[12] Approximately 65% of CdLS patients with clinical diagnosis carry mutations in one of cohesin-associated genes (*NIPBL, SMC1A* or *SMC3*).[13]

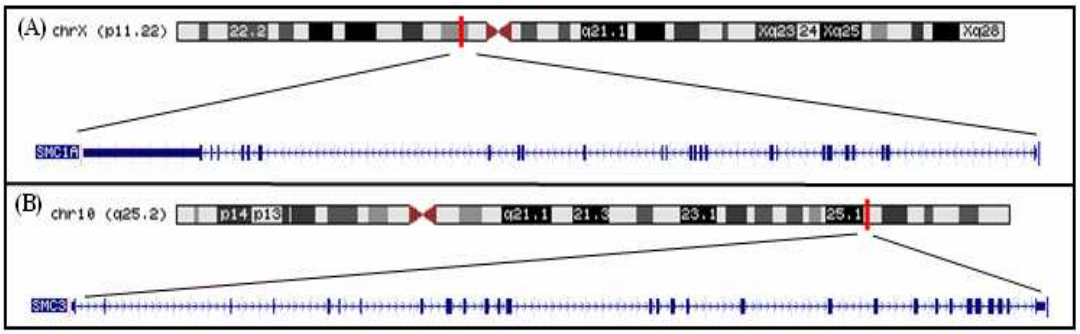

Figure 2. Genetic map of *SMC1A* (A) and *SMC3* (B) gene. (A) *SMC1A* gene is located on chromosome Xp11.22-p11.21 and contains 25 exons. (B) *SMC3* gene is located on chromosome 10q25.

Recently, chromosomal anomalies with translocation (t(14;21)(q32;q11), t(X;8)(p11.2;q24.3), and t(3;17)(q26.3;q23.1)),[14] and a duplication (0.6-Mb de novo 9p duplication)[15] were found in CdLS patients. These results suggest genetic heterogeneity of CdLS. Therefore, our study is designed to observe genetic heterogeneity of Korean patients with CdLS by mutation screening of *NIPBL* and *SMC1A* genes. Patients with no detectable mutation of *NIPBL* and *SMC1A* genes by sequencing were further investigated

Figure 3. Schematic organization of SMC proteins. They contain five distinct domains: N-terminal (or P-loop NTPase), a hinge motif flanked by two coiled coil regions and a C-terminal (or P-loop NTPase).

Table 1. Summary of reported mutations of *NIPBL*, *SMC1A* and *SMC3* gene in Cornelia de Lange Syndrome (Human Gene Mutation Database 2009)

***NIPBL* Mutation type**	**Total number of mutations**	***NIPBL* Mutation type**	**Total number of mutations**
Missense/nonsense	62	Small indels	2
Splicing	23	Gross deletions	1
Regulatory	1	Gross insertions	5
Small deletions	33	Complex rearrangements	1
Small insertions	19	Repeat variations	0
TOTAL			**147**

using whole genome array-based comparative genomic hybridization (array-CGH) in order to detect chromosome imbalances. Array-CGH uses two genomes, a test and a reference, which are differentially labeled with a fluorescent dye and competitively hybridized to a microarray.[16] The resulting ratio of the fluorescence intensities on each array element is proportional to the copy number of the corresponding DNA sequence in the test and reference genomes.[16]

Table 2. Summary of reported mutations of *SMC1A* and *SMC3* gene in Cornelia de Lange Syndrome (Human Gene Mutation Database 2009)

SMC1A **Mutation type**	**Total number of mutations**
Missense/nonsense	16
Small deletions	5
Gross insertions	1
complex rearrangements	1
TOTAL	**23**
SMC3 **Mutation type**	**Total number of mutations**
Small deletions	1 (c.1464_1466del3)
TOTAL	**1**

Array-CGH allows the mapping of genomic copy number alterations at the submicroscopic level, thereby directly linking disease phenotypes to gene dosage alterations.[16] Just as the t(5;13)(p13.1; q12.1) translocation paved the way for determining the *NIPBL* gene[5], other chromosomal alterations such as submicroscopic imbalances in different genomic regions could help the recognition and screening of novel candidate CdLS genes.

II. MATERIAL AND METHOD

1. Subjects and DNA extraction.

A total of 13 patients with CdLS phenotype were recruited from Yonsei University Hospital. Informed consent was obtained from the parents of the 13 patients in advance, before collecting blood samples. Genomic DNA was extracted from peripheral blood lymphocytes using Gentra Puregene Blood Kit (Qiagen, Valencia, CA, USA) in accordance to the manufacturer's protocol.

2. *NIPBL* and *SMC1A* gene mutation analysis.

The entire *NIPBL* gene except the first non-coding exon was screened for mutations in all tested patients, and those who did not have mutation in *NIPBL* were further analyzed for mutations in *SMC1A*. The *NIPBL* gene (exon 2 ~ 47) and *SMC1A* gene (exon 1 ~ 25) was amplified by PCR using TAKARA PCR Thermal Cycler DICE TP600 (TAKARA, Shiga, JAPAN). The RefSeqs, NM_133433 and NM_006306, obtained from University of California Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/ cgi-bin/hgGateway) were used as the reference sequence of *NIPBL* and *SMC1A*, respectively. PCR primers of *NIPBL* and *SMC1A* gene were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/, Steve Rozen and Helen J. Skaletsky (2000)) to amplify all exons and exon/intron boundaries. The coding regions of the *NIPBL* and *SMC1A* gene were amplified in a 30 reaction volume containing 100ng of genomic DNA, 10X SP-Taq reaction buffer, 0.75 unit SP-Taq polymerase(COSMO Genetech. Co.,LTD, Seoul, Korea), 10pmol of each primer pair, and 0.17mM of each dNTP. Cycling condition for *NIPBL* gene was as follows: a first denaturation 5min, 35 cycles at 94℃ for 30s, at 60℃ for 30s, and at 72℃ for 1min, with a last extension step at 72℃ for 7min. Cycling condition for *SMC1A* gene was as follows: a first denaturation 5min, 35 cycles at 94℃ for 30s, at 60℃ for 30s, and at 72℃ for 30s, with a last extension step at 72℃ for 7min. Each fragment amplified by PCR was sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on ABI PRISM 3100 Genetic Analyzer

(Applied Biosystems, Foster City, CA, USA). Sequence analysis was performed using ABI Sequence Scanner Software version 1.0 (Applied Biosystems, Foster City, CA, USA).

3. Array-CGH

Whole genome array-CGH was performed in patients who did not show any mutations within *NIPBL* and *SMC1A* gene in order to screen for genomic alterations. Agilent SurePrint G3 human CGH microarray was used with the resolution of 4x180K (Agilent Technologies Santa Clara, CA) containing approximately 180,000 oligonucleotides per 1 array, covering the genome with an average distance of 17kb. Array-CGH was performed by a commercial company, DNA Link, Inc.

III. RESULTS

1. Sequence analysis

The following five novel mutations within the *NIPBL* gene were identified in 13 patients: two frame shift mutations by deletion (c.6679-6682delGTCA, c.7549delG) and three missense mutations (c.2108C>A (p.Pro703Gln), c.6425G>T (p.Arg2142Ile), c.6530T>C (p.Leu2177Ser)). Two frameshift mutations, c.6679-6682delGTCA and c.7549delG in exon 39 and exon 44, cause premature termination codon at 2nd amino acid downstream in c.6679-6682delGTCA and 43rd amino acid downstream in c.7549delG. In addition, a number of single nucleotide polymorphisms were found in *NIPBL*. They are seven previously reported CdLS-related SNPs (c.2021A>G (p.Asn674Ser), c.3304+5G>T, c.3568-17A>G, c.3855+52A>G, c.4239+53T>G, c.5874C>T, c.4920-59G>A), two in dbSNP (c.3304+5G>T (rs80358356), c.3568-17A>G (rs78827246)), and four novel intronic SNPs (c.-112T>C, c.64-21A>C, c.6108+7A>G, c.7410+95C>T) (Fig 4).

Mutation analysis *SMC1A* in 8 patients without *NIPBL* gene mutation was performed, and one novel mutation, c.2327T>C (p.Val776Ala), was identified in a female patient. The fact that this mutation was not detected in her parents suggests this is a de novo mutation (Fig 5). Additionally, one novel silent mutation (c.579G>A) and three previously reported polymorphisms (c.-19C>T (dbSNP1264011), c.1337-32C>A (dbSNP1264008), c.2196-5T>C (dbSNP2297104)) were observed (Fig 6).

2. Array-CGH on patients without *NIPBL* and *SMC1A* mutations

Array-CGH was performed in 7 patients without mutations in either *NIPBL* or *SMC1A*. Two of these patients showed DNA copy number alterations including a deletion on chromosome 5q12.1 and a duplication on chromosome 16p11.2 of 37 Kb. The known genes mapping within the deleted and duplicated area are *NDUFAF2* and *ITGAX, ITGAD*, respectively (Fig 7).

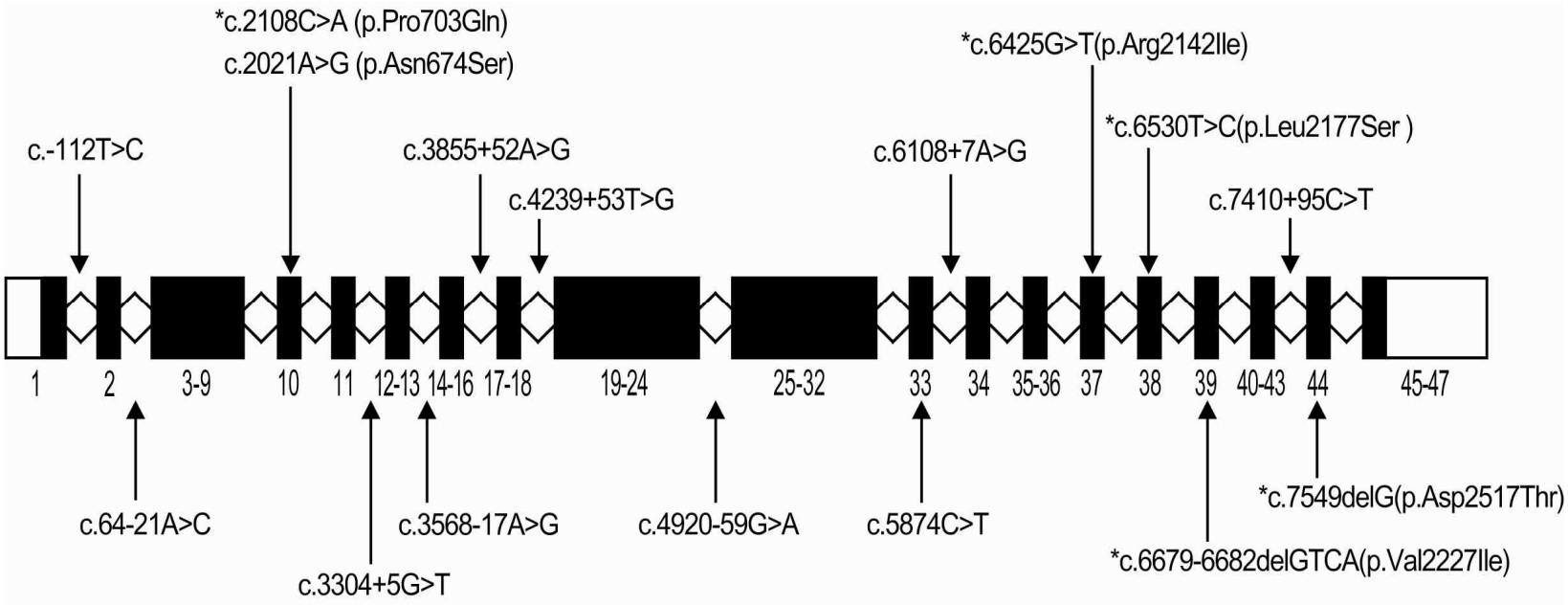

Figure 4. Five novel mutations and eleven polymorphisms in *NIPBL* gene identified from the 13 unrelated CdLS patients in this study. The star(*) indicates mutation, and the rest are polymorphisms. Labeled blocks represent exons.

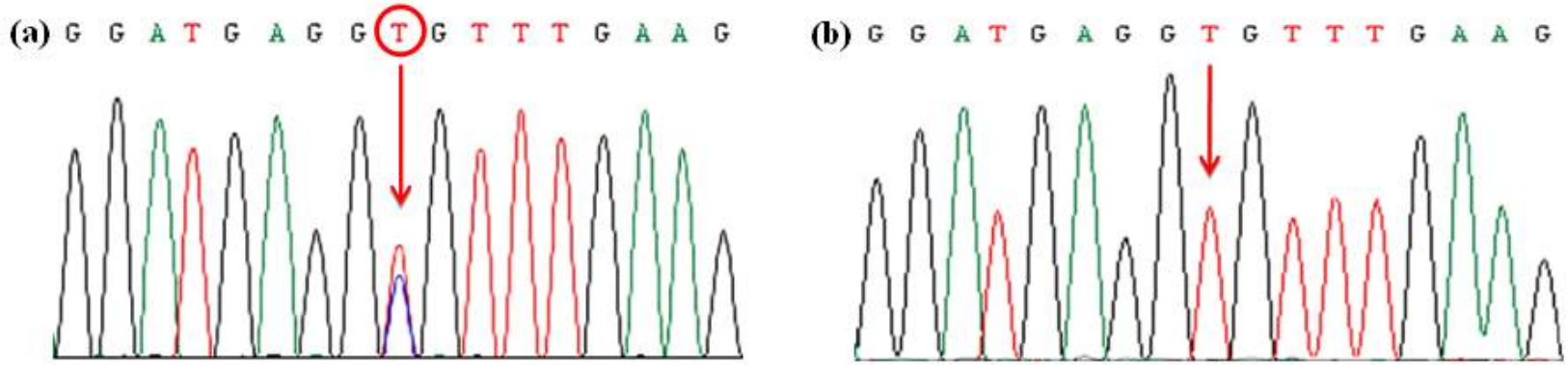

Figure 5. A heterozygous mutation (c.2327T>C (p.Val776Ala)) identified in *SMC1A* in a female patient (a) and her mother (b). The fact that this mutation was not detected in her parents indicates a de novo event.

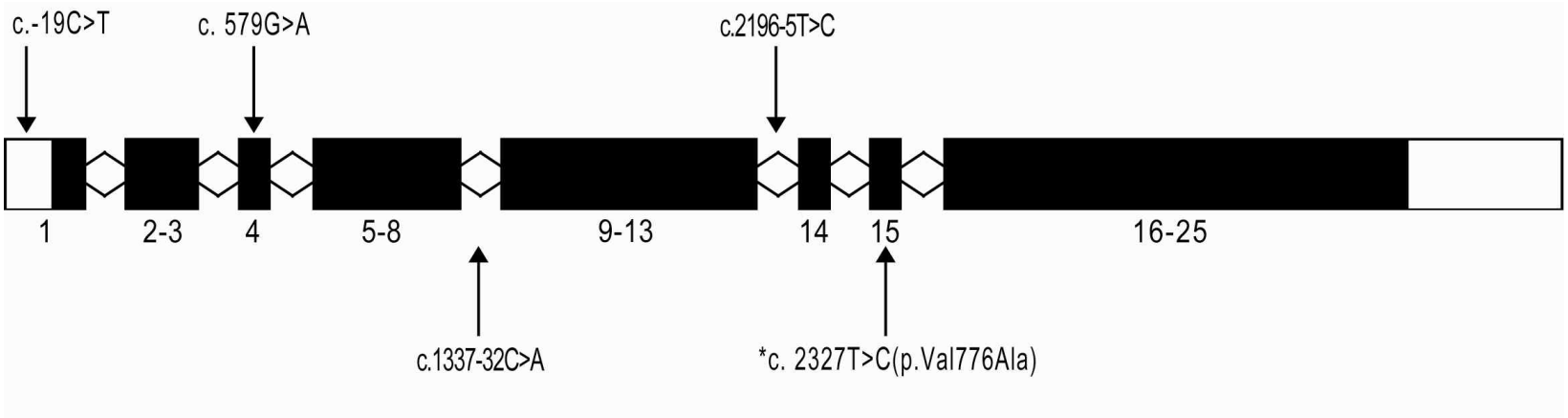

Figure 6. One novel mutation and four polymorphisms in *SMC1A* identified from the 8 unrelated CdLS patients who did not have mutations in *NIPBL*. The star(*) indicates mutation and the rest are polymorphisms. Labeled blocks represent exons.

Table 3. Summary of mutations in *NIPBL* and *SMC1A* genes identified in patients with CdLS

GENE	Exon Location	Base Change	Amino acid Change	Effect on Protein	Type of Mutation
NIPBL	Exon 10	c.2108C>A	p.Pro703Gln	-	Missense
	Exon 37	c.6425G>T	p.Arg2142Ile	-	Missense
	Exon 38	c.6530T>C	p.Leu2177Ser	-	Missense
	Exon 39	c.6679-6682delGTCA	p.Val2227Ile	Truncates protein 2 amino acid downstream	Frameshift
	Exon 44	c.7549delG	p.Asp2517Thr	Truncates protein 43 amino acid downstream	Frameshift
SMC1A	Exon 15	c. 2327T>C	p.Val776Ala	-	Missense

Table 4. Polymorphisms in *NIPBL* and *SMC1A* genes identified in 13 patients with CdLS

Gene	Polymorphism	Located in Exon	Reference
NIPBL	c.-112T>C	intron 1	Novel
	c.64-21A>C	intron 2	Novel
	c.2021A>G (p.Asn674Ser)	exon 10	Am. J. Hum. Genet. 75:610–623, 2004
	c.3304+5G>T	intron 11	rs80358356
	c.3568-17A>G	intron 13	rs78827246
	c.3855+52A>G	intron 16	Am. J. Hum. Genet. 75:610–623, 2004
	c.4239+53T>G	intron 18	Am. J. Hum. Genet. 75:610–623, 2004
	c.5874C>T	exon 33	Am. J. Hum. Genet. 75:610–623, 2004
	c.6108+7A>G	intron 34	Novel
	c.7410+95C>T	intron 43	Novel
	c.4920-59G>A	intron 24	Am. J. Hum. Genet. 75:610–623, 2004
SMC1A	c.-19C>T	5' UTR	dbSNP1264011
	c. 579G>A	exon 4	Novel
	c.1337-32C>A	intron 8	dbSNP1264008
	c.2196-5T>C	intron 13	dbSNP2297104

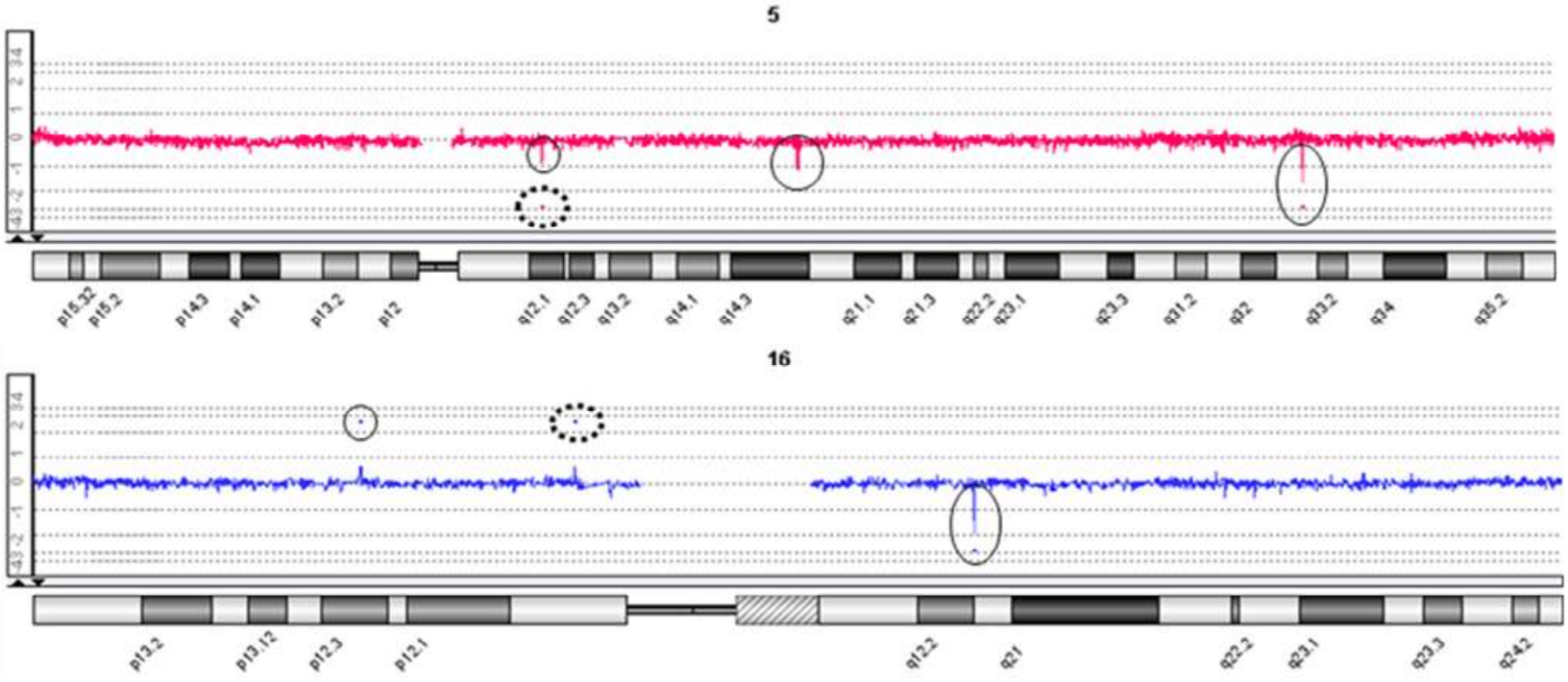

Figure7. Array-CGH profile of chromosomes 5 and 16. Deleted and duplicated regions are indicated by dotted circles. The regions indicated by solid circles correspond to known copy number variants. Chromosome deletion region 5q12.1 and duplication region 16p11.2 contain *NDUFAF2* and *ITGAX, ITGAD*, respectively.

IV. DISCUSSION

Two genes responsible for CdLS are *NIPBL*, a regulator of the cohesin complex, that was discovered in a patient with t(5;13)(p13.1;q12.1) translocation,[5] and *SMC1A*, a core component of the cohesin complex.[7] The mutation detection rates in *NIPBL* and *SMC1A* genes were 38% and 9%, respectively, similar to the rate previously reported in a study on Caucasian patients.[17] A total of six different mutations were identified which are two frameshift mutations, three missense mutations in *NIPBL,* and one missense mutation in *SMC1A* gene.

According to a study by Gillis's (2004), there is no mutation in exons 4–6, 8, 11–14, 16, 19, 23–25, 30–34, 36, 37, 41, and 47.[14] Also Mutations identified in *NIPBL* gene show predominance in exon 10.[14] Mutations identified in this study were located in exon 10, 37, 38, 39, and 40. Both frameshift mutations discovered in *NIPBL* are expected to cause prematurely truncated protein products, likely resulting in haploinsufficiency of *NIPBL* gene, which is a key mechanism in CdLS. Four missense mutations detected in *NIPBL* and *SMC1A* can cause the disease by changing a protein residue.

SMC1A is located in the X chromosome, and is reported to escape X inactivation in human.[8] In a female patient with a heterozygous mutation, normal and mutant alleles did not show any significant difference in the production of *SMC1A* mRNA. Therefore, a carrier female with a mutation in *SMC1A* gene may show symptoms of CdLS by the dominant negative effect.[18] Most of the reported *SMC1A* mutations are not located in the amino terminal NTPase domain from amino acid 4–148, the hinge domain from amino acid 515–629, or the carboxy terminal NTPase domain from amino acid 1,117–1,220. This indicates that chromosome cohesion may not play a major role in the pathogenesis of CdLS.[18] In our study, a heterozygous mutation identified in a female patient is c.2327T>C (p.Val776Ala) in exon 15, which that is not an ATPase domain or a hinge domain.

Also we found two carriers of DNA copy number alterations, which include a deletion on chromosome region 5q12.1 and a duplication on chromosome region 16p11.2 both of

length 37 kb. The deleted area contains the gene *NDUFAF2* that encodes mitochondrial complex 1 assembly factor.[19] Mutations in this gene cause progressive encephalopathy.[20] The duplicated area contains genes ITGAX and ITGAD which encode integrin alpha-X protein and integrin alpha-D protein, respectively.[21] These proteins are composed of integrin receptors involved in inflammatory response, monocyte adhesion, chemotaxis, and phagocytosis.[22] The results of array-CGH have no immediately apparent relationship to CdLS.

In order to explain the correlation between causative gene mutations and all patient phenotypes, further work is needed to find additional candidate genes. We are currently working to discover new causative genes associated with CdLS by whole exome sequencing.

V. CONCLUSION

We identified six novel mutations in 13 unrelated Korean patients clinically diagnosed with CdLS. Those mutations will promote understanding of genetic background in Korean patients with CdLS for the future and will used be to construct a Korean CdLS mutation database. Approximately 46% of Korean patients with CdLS in this study have these mutations, which is similar to the rate of another study (Juan Pie journals: NIPBL:47%, SMC1A:10%), strongly suggests the genetic heterogeneity of this syndrome.

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ABSTRACT (IN KOREAN)

한국인 Cornelia de Lange 증후군 환자의 NIPBL 유전자 돌연변이 분석

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목적: Cornelia de Lange 증후군은 성장지연, 정신지체의 이상소견과 특징적인 얼굴모습을 동반한 선천성 다발성 기형 증후군이다. 현재까지 약 50%의 환자에서 NIPBL, SMC1A 유전자 변이와 연관된 것으로 알려져 있어 질환의 유전적 배경으로 유전적 이질성(genetic heterogeneity) 현상을 추정할 수 있다. 따라서 한국인 Cornelia de Lange 증후군 환자들을 대상으로 원인 유전자들의 돌연변이 분석을 시행하고, genome 상의 결손과 중복 분석을 통해 새로운 후보 유전자를 검색하고자 한다.

대상 및 방법: 연세대학교 신촌 세브란스 병원 임상유전학과에서 Cornelia de Lange 증후군으로 진단되어 추적중인 13명의 한국인 환자를 대상으로 유전자 검사를 시행하였다. 돌연변이 분석은 직접염기서열 분석으로 시행하였고, genome 상의 결손이나 중복은 array-based comparative genomic hybridization

(array-CGH) 방법으로 확인하였다.

결과: 13명의 환자 중 5명의 환자에서 NIPBL 유전자 돌연변이가 발견되었으며, 1명의 환자에서 SMC1A 유전자 돌연변이가 발견되어 총 6명의 환자에서 유전자 돌연변이가 보고되었다. NIPBL 유전자에서 발견된 돌연변이는 c.2108C>A (p.Pro703Gln), c.6425G>T (p.Arg2142Ile), c.6530T>C (p.Leu2177Ser), c.6679-6682delGTCA, c.7549delG이며, SMC1A 유전자에서 발견된 돌연변이는 c.2327T>C (p.Val776Ala)로 현재까지 보고되지 않은 돌연변이이다. 이들 유전자에서 돌연변이가 발견되지 않은 환자 7명을 대상으로 실시한 array-CGH 결과 2명의 환자에서 비정상적인 염색체의 양상이 발견되었다.

결론: 본 연구에서 새롭게 발견된 돌연변이는 향후 한국인 Cornelia de Lange 증후군의 유전적 진단에 있어 유용한 자료가 될 것으로 사료되지만 array-CGH 결과 발견된 비정상적인 부의의 유전자 조사 결과 이 증후군과 특이한 상관관계는 없는 것으로 생각된다. 따라서 이 증후군의 유전적 이질성을 이해하여 새로운 후보 유전자를 발굴하기 위한 연구가 필요하다.

핵심 되는 말: Cornelia de Lange 증후군, NIPBL, SMC1A, array-based comparative genomic hybridization, 유전적 이질성