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MUTATION IN BRIEF

Incidence and Clinical Features of X-linked Cornelia de Lange Syndrome Due to *SMC1L1* Mutations

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Cornelia de Lange syndrome (CdLS) is a multisystem developmental disorder characterized by facial dysmorphism, growth and mental retardation, microcephaly, and various malformations. Heterozygous mutations in the *NIPBL* gene have been detected in approximately 45% of affected individuals. Recently, a second CdLS gene, mapping to the X chromosome, has been identified: *SMC1L1* (*structural maintenance of chromosomes 1-like 1*; or *SMC1A*). In order to estimate the incidence and refine the clinical presentation of X-linked CdLS, we have screened a series of 11 CdLS boys carrying no *NIPBL* anomaly. We have identified two novel de novo *SMC1L1* missense mutations (c.587G>A [p.Arg196His] and c.3254A>G [p.Tyr1085Cys]). Our results confirm that *SMC1L1* mutations cause CdLS and support the view that *SMC1L1* accounts for a significant fraction of boys with unexplained CdLS. Furthermore, we suggest that *SMC1L1* mutations have milder effects than *NIPBL* mutations with respect to pre- and postnatal growth retardation and associated malformations. If confirmed, these data may have important implications for directing mutation screening in CdLS.

KEY WORDS: Cornelia de Lange syndrome; *SMC1L1*; *NIPBL;* growth retardation; genotype-phenotype correlation

INTRODUCTION

Cornelia de Lange syndrome (CdLS; also called Brachmann de Lange syndrome; MIM# 122470) is a rare developmental disorder characterized by facial dysmorphism, pre- and postnatal growth retardation with microcephaly, mental retardation, absent or delayed speech, and various malformations, including upper limb defects (Ireland et al., 1993; Jackson et al., 1993). Mutations in a major CdLS disease gene, the *NIPBL* gene (*Nipped-B homolog (Drosophila)*; MIM# 608667) are detected in approximately 45% of affected individuals (Gillis et al., 2004; Krantz et al., 2004; Tonkin et al., 2004). *NIPBL* maps to chromosome 5p13.2 and encodes delangin, the mammalian ortholog of Drosophila Nipped-B and yeast Scc2 which are both involved in sister chromatid cohesion by loading cohesin complexes onto chromatin (Strachan, 2005; Dorsett, 2006). CdLS-causing mutations are scattered throughout this large gene and include nonsense, frameshift, splice site, and missense

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mutations, as well as a mutation in the 5'untranslated region (5'UTR) and small in-frame deletions (Borck et al., 2004; Gillis et al., 2004; Krantz et al., 2004; Tonkin et al., 2004; Miyake et al., 2005; Bhuiyan et al., 2006; Borck et al., 2006; Yan et al., 2006). Absence of *NIPBL* mutations in half of affected persons suggested genetic heterogeneity in CdLS.

A second disease gene has been recently identified. Indeed, studying a family in which CdLS was apparently transmitted as an X-linked trait, Musio et al. (2006) detected mutations in the *SMC1L1* gene in three affected males from one family and subsequently in 1/32 sporadic CdLS cases with no *NIPBL* mutation. *SMC1L1* (*structural maintenance of chromosomes 1-like 1*, or *SMC1A*; MIM #300040) maps to chromosome Xp11.22 and encodes one of the four cohesin subunits which hold sister chromatids together from S phase until the beginning of anaphase (Nasmyth and Haering, 2005). *SMC1L1* has also been shown to be involved in genome stability and DNA repair (Musio et al., 2005; Watrin and Peters, 2006). The phenotype of X-linked CdLS (X-CdLS; MIM #300590) in the four reported males seemed to differ from the typical form by a milder dysmorphism, mild or no growth retardation and absence of microcephaly (Musio et al., 2006).

In order to estimate the frequency and refine the clinical presentation of X-CdLS, we have screened the *SMC1L1* gene in a series of 11 CdLS boys with no identifiable *NIPBL* anomaly. Our results show that a significant proportion of these CdLS boys carried *SMC1L1* mutations (2/11). The *SMC1L1* mutations were associated with a typical CdLS dysmorphism but affected boys had milder growth retardation as compared to children carrying *NIPBL* mutations.

PATIENTS, MATERIALS, AND METHODS

Patients

The present series included 11 unrelated boys ranging in age from one to 20 years. All patients were followed at the Department of Genetics of the Necker-Enfants Malades Hospital, Paris. They were diagnosed with CdLS on the basis of four major criteria: (i) pre- and/or postnatal growth retardation, (ii) pre- and/or postnatal microcephaly, (iii) moderate to profound mental retardation with abnormal speech development, ranging from minimal speech to complete absence of speech, and (iv) facial dysmorphic features including arched eyebrows with synophrys, short nose with anteverted nares, long philtrum, and thin upper lip. In addition, at least two of the following four clinical signs or symptoms were required for diagnosis, namely: (i) behavioral problems including self-injurious behavior, hyperactivity, repetitive and autistic behavior, (ii) feeding problems in infancy and/or gastroesophageal reflux, (iii) major or minor anomalies of the upper limbs, and (iv) skin anomalies, particularly hirsutism and cutis marmorata.

All subjects were also evaluated for CdLS-associated major malformations such as cleft palate and cardiac, gastrointestinal, genitourinary, and renal anomalies.

Molecular Studies

Blood samples were obtained from the probands and their parents after written informed consent. All patients had been tested for mutations in the 46 *NIPBL* coding exons and the 5'UTR exon 1 by direct sequencing. Partial or whole-gene *NIPBL* deletions had been excluded by multiplex ligation-dependent probe amplification (MLPA kits P141 and P142, MRC-Holland, Amsterdam, The Netherlands; www.mrc-holland.com; Schouten et al., 2002).

The *SMC1L1* gene was submitted to PCR amplification of the 25 exons and exon-intron boundaries from genomic DNA (accession number NM_006306.2) using previously reported primer pairs (Musio et al., 2006), except for exon 19 which was amplified using primers 19F-5'-CAAAGCCTCTGTTCCTTCTG-3' and 19R-5'-TCTTTCCAGCCATGGTCTTC-3'. PCR products were treated with Exo-SAP-It (Amersham Biosciences, Orsay, France; www5.amershambiosciences.com) and directly sequenced in both directions on an ABI 3130 sequencer using the dye terminator method according to the manufacturer's instructions (Applied Biosystems, Courtaboeuf, France; www.appliedbiosystems.com). The two identified mutations were detected on two separate DNA samples each. Segregation of ten polymorphic microsatellite markers confirmed regular biparental inheritance in both cases. Nomenclature of the *NIPBL* and *SMC1L1* mutations is based on their position in the respective mRNA sequence with the A of the translation initiation codon being nucleotide +1.

Genotype-Phenotype Correlation Studies

For genotype-phenotype correlations, *NIPBL* mutations were classified as truncating (nonsense, frameshift and splice-site mutations) or non-truncating (missense and 5'UTR mutations and an in-frame deletion). The 13 *NIPBL* mutations included six previously reported (Borck et al., 2004, 2006) and seven unreported mutations (reference sequence NM_133433.2): c.64+5G>A, c.65-5A>G, c.4643+2T>G, c.5455G>T (p.Arg1819X), c.5671A>G (p.Lys1891Glu), c.6166C>T (p.Pro2056Ser) and c.7189T>C (p.Ser2397Pro).

For comparison of continuous variables (weight, length and occipitofrontal circumference [OFC] at birth) between *NIPBL*- and *SMC1L1*-mutation positive groups, the Wilcoxon rank sum test was used. Qualitative variables (percentage of patients with major malformations and seizures) were compared using Fisher's exact test. Tests with p-values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

We screened the *SMC1L1* gene in 11 CdLS boys with no identifiable *NIPBL* anomaly and we identified mutations in two patients. Patient 1 had a c.3254A>G transition in exon 21 which is predicted to lead to a p.Tyr1085Cys missense change. Patient 2 was shown to have a c.587G>A transition in exon 4 predicting a p.Arg196His missense change. Both substitutions are likely disease-causing as they affected amino acid residues that are highly conserved in a wide range of SMC1L1 orthologs and homologs (Fig. 1). Moreover, these base changes were not identified in parental DNA, suggesting *de novo* events, and they were not detected in 120 control X chromosomes.

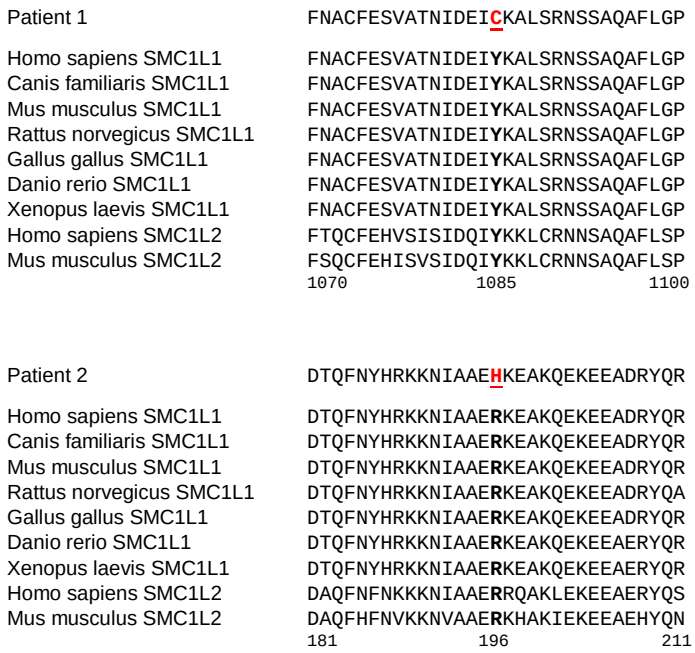

Figure 1. Evolutionary conservation of amino acid residues altered by missense substitutions in SMC1L1. The polypeptide sequences surrounding the mutated amino acids (reference sequence NP_006297) were aligned to homologous SMC1L1 and SMC1L2 sequences (www.ncbi.nih.gov/blast).

The two boys carrying a *SMC1L1* mutation had the following growth parameters at birth: patient 1, weight 2400 g, length 45 cm and OFC 31 cm (all parameters <5th centile for gestational age) and patient 2, weight 3580 g (50th-90th centile), length 49 cm (10th-50th centile) and OFC 32 cm (<5th centile). At age six years, patient 1's height was 104 cm (-2.1 SD), weight 16 kg (-1.8 SD) and OFC 47.5 cm (-2.5 SD). At age two years five months, growth retardation persisted in patient 2 with height 81 cm (-2.7 SD), weight 9.5 kg (-2.8 SD) and OFC 43.5 cm (-4.2 SD). The two boys had typical CdLS with developmental delay, feeding problems and gastroesophageal reflux, severe speech delay, short feet and hands with brachydactyly V, hirsutism, and facial dysmorphic features

(Fig. 2). In addition, patient 1 had recurrent otitis media, myopia, strabismus, cutis marmorata, repetitive and auto-aggressive behavior and febrile seizures. None of the boys had major malformations. The mothers of Patients 1 and 2 had normal growth parameters and cognitive function and were not dysmorphic.

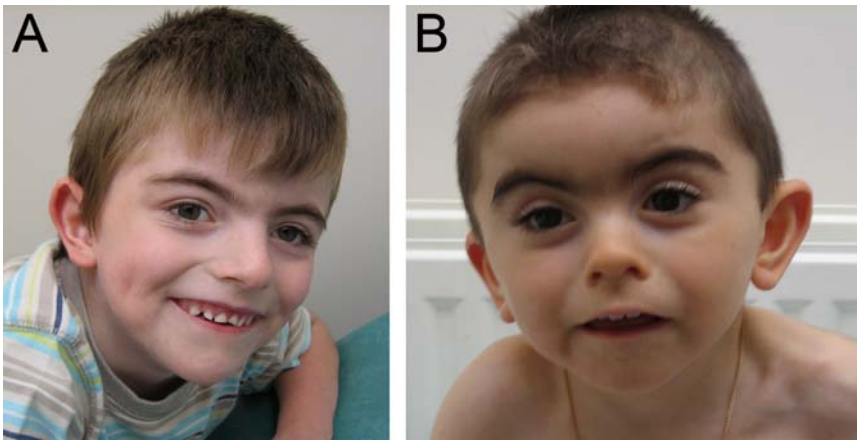

Figure 2. Photographs of patients 1 (**A**) and 2 (**B**) showing dysmorphic features of CdLS caused by *SMC1L1* mutations. Note synophrys, long eyelashes, thin lips and widely spaced teeth in Patient 1. Patient 2 has arched eyebrows with synophrys, long eyelashes, short nose with anteverted nares, thin upper lip and downturned corners of the mouth. Photographs are reproduced with permission.

The SMC (structural maintenance of chromosomes) superfamily proteins contain ATP-binding domains at the N- and C-termini and two extended coiled-coil domains separated by a hinge region. Interestingly, the p.Tyr1085Cys substitution identified in the more severely affected boy (patient 1) altered an amino acid located in the C-terminal ATP-binding domain of the protein which confers ATPase activity (Arumugam et al. 2003; Weitzer et al., 2003). By contrast, the amino acid mutated in the less severely affected patient 2 (Arg196) did not lie in any known functional domain of the SMC1L1 protein.

These results support the view that *SMC1L1* is a disease-causing gene in CdLS. In their study, Musio et al. (2006) identified *SMC1L1* mutations in 2/33 CdLS index patients of both sexes. The figure was even higher in our series, as 2/11 boys were found to carry hitherto unreported *SMC1L1* mutations. Considering that our series consisted of 30 affected individuals in whom we identified 13 distinct *NIPBL* mutations (Borck et al., 2004, 2006, and unpublished results), our data suggest that *SMC1L1* mutations account for at least 2/30 cases. It is worth noting also that no *SMC1L1* mutations were found in 9/11 boys, strongly suggesting the existence of at least one other locus in CdLS.

In order to contribute to the study of genotype-phenotype correlations and to hopefully direct mutation search in CdLS, we compared the clinical features of six patients with a *SMC1L1* mutation (Patients 1 and 2 from this report and the four previously reported boys [Musio et al., 2006]) with those of 14 individuals harboring *NIPBL* mutations (eight boys and six girls). The results are shown in Table 1. No phenotype was specifically associated with either *NIPBL* or *SMC1L1* mutations. Yet, several differences between the two groups could be noted. First, X-CdLS apparently caused less severe pre- and postnatal growth retardation, as patient 2 had a normal length and weight at birth. Moreover, 3/4 affected males reported by Musio et al. (2006) had no postnatal growth retardation and none of them had microcephaly. It is worth noting that these differences in growth parameters were not due to sex ratio differences as they persisted when X-CdLS patients were compared to boys carrying a *NIPBL* mutation (Table 1). Second, significantly more frequent seizures (mostly febrile) and significantly fewer major malformations were noted in X-CdLS. Interestingly, the only major malformation reported to date in X-CdLS was plagiocephaly (Musio et al., 2006), a malformation that is not commonly seen in CdLS. Although sample sizes are small, these differences are interesting to note and will hopefully be confirmed in larger studies.

Table 1. Results of Genotype-Phenotype Correlation Analysis in CdLS Patients Carrying *SMC1L1* or *NIPBL* Mutations

	SMC1L1	***NIPBL***			
Type of mutation	Missense	Total All 7 nontruncating 6 truncating	Nontruncating	Truncating	Boys only all 4 nontruncating 4 truncating
Number of mutations	4	14	7	6	8
Number of patients (♂/♀)	6 ♂ [1]	14 (8 ♂ / 6 ♀) [2]	8 (4 ♂ / 4 ♀)	6 (4 ♂ / 2 ♀)	8 ♂
Birth weight in g (±SD)	2608* (±601)	2257 (±420)	2310 (±445)	2187 (±413)	2204 (±492)
Length at birth in cm (±SD)	47.0** (±2.8)	43.4 (±3.0; n=12)	43.3 (±3.2)	43.6 (±2.9; n=4)	42.3 (±2.7; n=7)
OFC at birth in cm (±SD)	31.9* (±0.5)	31.0 (±1.6; n=11)	31.3 (±1.4)	30.3 (±2.1; n=3)	30.8 (±1.5; n=5)
Postnatal height (±SD)	-2.4 (±0.4; n=2)	-3.1 (±1.3)	-2.8 (±0.7)	-3.6 (±1.8)	-2.8 (±1.0)
Postnatal weight (±SD)	-2.3 (±0.7; n=2)	-2.2 (±1.2)	-2.0 (±0.9)	-2.5 (±1.6)	-2.1 (±1.1)
Postnatal OFC (±SD)	-3.4 (±0.9; n=2)	-3.6 (±1.3; n=13)	-3.1 (±0.9; n=7)	-4.3 (±1.5)	-3.9 (±1.5; n=7)
Major malformations (number of malformations/ patient with a malformation)	1/6** (1.0)	10/13 (1.6)	5/7 (1.2)	5/6 (2.0)	7/8 (1.7)
Seizures	5/6***	2/13	1/7	1/6	1/8

Standard deviations (SD) are given in parentheses.

[1] Six male patients from four families (mutations: p.Asp831Glu/Gln832del, p.Glu493Ala [Musio et al., 2006], p.Tyr1085Cys and p.Arg196His [this report])

[2] Fourteen patients with 13 distinct mutations (one familial case); mutations reported in Borck et al. (2004, 2006) and in this report

* not significant ($p>0.05$) for the comparison of patients with *SMC1L1* versus *NIPBL* mutations

** $p<0.05$ for the comparison of patients with *SMC1L1* versus *NIPBL* mutations

*** $p<0.01$ for the comparison of patients with *SMC1L1* versus *NIPBL* mutations

In conclusion, we suggest that the *SMC1L1* gene should be screened in boys with unexplained CdLS. Based on our observations, we suggest that mutations in *SMC1L1* might cause a milder form of the syndrome. Hence, normal growth parameters at birth might prompt one to first screen for *SMC1L1* mutations.

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