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Precocious Sister Chromatid Separation (PSCS) in Cornelia de Lange Syndrome

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Abstract

The Cornelia de Lange syndrome (CdLS) (OMIM# 122470) is a dominantly inherited multisystem developmental disorder. The phenotype consists of characteristic facial features, hirsutism, abnormalities of the upper extremities ranging from subtle changes in the phalanges and metacarpal bones to oligodactyly and phocomelia, gastroesophageal dysfunction, growth retardation, and neurodevelopmental delay. Prevalence is estimated to be as high as 1 in 10,000. Recently, mutations in *NIPBL* were identified in sporadic and familial CdLS cases. To date, mutations in this gene have been identified in over 45% of individuals with CdLS. *NIPBL* is the human homolog of the *Drosophila Nipped-B* gene. Although its function in mammalian systems has not yet been elucidated, sequence homologs of Nipped-B in yeast (*Scc2* and *Mis4*) are required for sister chromatid cohesion during mitosis, and a similar role was recently demonstrated for Nipped-B in *Drosophila*. In order to evaluate *NIPBL* role in sister chromatid cohesion in humans, metaphase spreads on 90 probands (40 *NIPBL* mutation positive and 50 *NIPBL* mutation negative) with CdLS were evaluated for evidence of precocious sister chromatid separation (PSCS). We screened 50 metaphases from each proband and found evidence of PSCS in 41% (compared to 9% in control samples). These studies indicate that *NIPBL* may play a role in sister chromatid cohesion in humans as has been reported for its homologs in *Drosophila* and yeast.

Keywords

cornelia de Lange syndrome; CdLS; *NIPBL*; *Nipped-B*; precocious sister chromatid separation; PSCS

Introduction

The Cornelia de Lange syndrome (CdLS), also termed the Brachmann de Lange syndrome (BDLS) (OMIM# 122470), is a dominantly inherited genetic multisystem developmental

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disorder. The phenotype consists of characteristic facial features (Fig. 1), hirsutism, abnormalities of the upper extremities (ranging from subtle changes in the phalanges and metacarpal bones to oligodactyly and phocomelia), gastroesophageal dysfunction, growth retardation, and neurodevelopmental delay [Ireland and Burn, 1993; Jackson et al., 1993]. Other frequently seen findings include ptosis, myopia, cryptorchidism, hypospadias, pyloric stenosis, congenital diaphragmatic hernias, cardiac septal defects, seizures, and hearing loss. The mental retardation seen in CdLS, although typically moderate to severe, does display a wide range of variability with IQs ranging from 30 to 86 with an average of 53.

Recently, mutations in *NIPBL* were identified in sporadic and familial CdLS cases. To date, mutations in this gene have been identified in over 45% of individuals with CdLS [Gillis et al., 2004]. *NIPBL* is the human homolog of the *Drosophila Nipped-B* gene. Although its function in mammalian systems has not been elucidated, Nipped-B has been shown to be an essential regulator of *cut*, *Ultrabithorax*, and Notch receptor signaling in *Drosophila*. Sequence homologs of Nipped-B in yeast (Scc2 and Mis4) are required for sister chromatid cohesion during mitosis, and a similar role was recently demonstrated for Nipped-B in *Drosophila* [Rollins et al., 2004]. In order to evaluate *NIPBL*'s role in sister chromatid cohesion in humans, metaphase spreads on a large cohort of mutation positive and mutation negative probands with CdLS were evaluated for evidence of precocious sister chromatid separation (PSCS). PSCS was seen in a significant number of CdLS probands when compared to unaffected controls. These studies indicate that *NIPBL* may play a role in sister chromatid cohesion in humans as has been reported for its homologs in *Drosophila* and yeast. The identification of PSCS in individuals with CdLS may be a helpful diagnostic aid, as *NIPBL* mutational analysis is currently only available on a research basis and mutations are identified in only ~45% of affected probands.

Materials and Methods

Cornelia de Lange Syndrome Patients

All individuals enrolled in the study were ascertained by the authors as having a diagnosis of CdLS. All patients and family members were enrolled in the study under an IRB-approved protocol of informed consent at The Children's Hospital of Philadelphia.

Chromosomal Analysis and Evaluation for PSCS

Metaphase spreads were prepared for the 90 CdLS probands and 90 controls without clinical features of CdLS, or other conditions known to be associated with PSCS, from either whole blood (cultured in RPMI 1640 with 15% fetal bovine serum and phytohemagglutinin for 72 hr) or lymphoblastoid cell lines (transformed with Epstein-Barr Virus and harvested during the log phase). Cells were arrested at metaphase with 0.8 µg/ml Colchicine (SIGMA-ALDRICH) for 20 min at 37°C, hypotonized with 0.075 M KCL at room temperature and fixed with three parts methanol: 1 part acetic acid. The slides were stained with Wright's Stain (Fisher Scientific). Ten proband slides were C-banded (constitutive heterochromatin). A minimum of 50 metaphases were microscopically examined and scored for PSCS. PSCS was diagnosed when the sister chromatids were completely separated and no connection at the centromere was seen [Plaja et al., 2003]. A metaphase was scored as positive for PSCS if all or the majority of sister chromatids in the metaphase spread demonstrated sister chromatid separation. A positive PSCS score was recorded for any individual with at least one metaphase per slide demonstrating PSCS.

Mutational Analysis Using Conformation Sensitive Gel Electrophoresis (CSGE)

Conformation sensitive gel electrophoresis (CSGE) was carried out using standard protocols [Ganguly et al., 1993]. Oligonucleotide primer sequences and PCR conditions used for

amplification of all exons of the *NIPBL* gene are as previously reported [Gillis et al., 2004]. PCR products corresponding to all altered migration patterns (shifts) were purified using QIAquick® PCR purification kit, (QIAGEN Sciences) and sequenced on an ABI 377 sequencer.

Results

In order to evaluate *NIPBL*'s role in sister chromatid cohesion in humans, metaphase spreads on 90 CdLS probands (40 *NIPBL* mutation positive and 50 *NIPBL* mutation negative) were evaluated for evidence of PSCS (Table I). We screened a minimum of 50 metaphases from each proband and found evidence of PSCS in 37 of 90 probands (41%) (Fig. 2). Of these 37 probands with PSCS, 16 (43%) were mutation positive and 21 (57%) mutation negative. Of the 53 probands without evidence of PSCS, 24 (45%) were mutation positive and 29 (54%) were mutation negative. Ninety control slides were screened and 8 (9%) demonstrated evidence of PSCS. Both severe and mild CdLS phenotypes were seen in the PSCS positive and negative groups. Missense, frameshift, and nonsense mutations have been seen in both groups. Two of the patients with PSCS have the same missense mutation: R2298H in exon 40 in a highly conserved amino acid residue. An average of 2.05 metaphases (4%) (range: 2%–10%) were found to have PSCS in the CdLS probands where as in the PSCS positive control samples an average of 1.125 metaphases (2%) (range 2%–4%) were found to have PSCS. All PSCS positive control samples had only one PSCS per sample except for one sample that had two. Results of PSCS screening from the CdLS cohort with *NIPBL* mutation status are listed in (Table II). Additionally, several metaphase spreads from individuals with CdLS demonstrated some evidence of breakage (Fig. 2I).

Discussion

In eukaryotic cells, replicated DNA molecules remain physically connected from their synthesis in S phase until they are separated during anaphase. This phenomenon, called sister chromatid cohesion, is essential for the temporal separation of DNA replication and mitosis and for the equal separation of the duplicated genome. Sister chromatids in metaphase chromosomes are physically connected until their separation during anaphase [Nasmyth et al., 2000, 2001]. The “glue” that holds sister chromatids together is a protein complex called “cohesin.” Cohesin is a four-member protein complex composed of a heterodimer of SMC1 and SMC3 associated with SCC1 and SCC3 proteins [Hagstrom and Meyer, 2003]. Several other proteins are involved in the loading, unloading, maintenance, and function of this complex. *NIPBL*, the gene that results in CdLS when mutated, codes for a protein that appears to be involved in the loading and unloading of the cohesin complex onto and off of the chromosomes [Rollins et al., 2004]. The inability to properly regulate the loading and unloading of the cohesin complex, which is regularly spaced on mammalian chromosomes at approximately 20 kb intervals, in CdLS appears to disrupt long-range enhancer–promoter interactions and likely is the cause of the main phenotypic findings in this disorder [Krantz et al., 2004; Rollins et al., 2004]. This study investigated whether or not the role of the cohesin complex in sister chromatid cohesion was also affected by alterations of *NIPBL* in CdLS probands. PSCS is a phenomenon whereby separate and splayed chromatids, with discernible centromeres, are seen and involves all or most chromosomes of a metaphase [Kajii and Asamoto, 2004]. It involves not only the centromere but also the entire sister chromatids of almost all mitotic chromosomes in a given metaphase [Kajii and Ikeuchi, 2004]. PSCS has been described in a number of conditions including Roberts syndrome [German, 1979], Fanconi Anemia, Ataxia Teleangiectasia [Mehes and Buhler, 1995], Alzheimer disease [Moorhead and Heyman, 1983; Spremo-Potparevic et al., 2004], Tuberous Sclerosis [Scappaticci et al., 1988], and Variegated Aneuploidy [Kajii et al., 1998; Plaja et al., 2001, 2003]. Recently, mutations in the *BUB1B* gene were found to be a cause of multiple variegated

aneuploidy [Hanks et al., 2004]. *BUB1B* encodes BUBR1, a key protein in the mitotic spindle checkpoint. PSCS has also been described in association with cancer, such as Wilms tumor [Mehes et al., 2002] and breast cancer [Rao et al., 1996]. PSCS has been seen in spontaneous abortions [Keser et al., 1996] and also in normal individuals after exposure to genotoxic chemicals [Major et al., 1999]. PSCS has also been reported to be present in a low percentage (less than 2%–3%) of normal individuals [Dominguez and Rivera, 1992; Kajii and Ikeuchi, 2004].

Due to the role-played by the yeast homologs of NIPBL (*Scc2*, *Rad21*, *Mis4*) in sister chromatid cohesion and the evidence that a similar cohesion abnormality is seen in *Drosophila* [Rollins et al., 2004], we hypothesized that a similar phenomenon may be present in individuals with CdLS. In studying a minimum of 50 metaphase spreads in each of 90 CdLS individuals and 90 control subjects, we found a significant increase in PSCS (41%) in the CdLS samples over control samples (9%) (P -value 0.0000003). The presence or absence of PSCS in CdLS did not appear to be influenced by the presence or absence of an identified mutation in *NIPBL* or by the age or sex of the individuals with CdLS.

The finding of several metaphases in some of the CdLS probands demonstrating apparently increased breakage (not observed in any of the controls) (Fig. 2I) indicates that there may be some predisposition to chromosomal fragility in CdLS probands; however, this has not been formally assessed as part of this study and warrants further investigation.

It is of interest that Roberts syndrome, an autosomal recessive condition that has phenotypic overlap with CdLS consisting of craniofacial anomalies, tetraphocomelia, cardiac septal defects with growth and cognitive retardation that has been classically associated with PSCS, was recently found to be caused by mutations in the *ESCO2* gene [Vega et al., 2005]. The *ESCO2* gene is a member of a conserved protein family required for the establishment of sister chromatid cohesion during the S phase of the cell cycle. This discovery implicates disruption of the cohesion complex in another human developmental disorder, with involvement of organ systems in a strikingly conserved pattern to that seen in CdLS.

The identification of PSCS in individuals with CdLS may be of value diagnostically. Presently mutations in *NIPBL* are identified in approximately 45% of individuals with a clear diagnosis of CdLS [Gillis et al., 2004; Krantz et al., 2004; Tonkin et al., 2004]. There has not been any clear evidence that there are other loci for a *CdLS* gene at this time and it may be that the low mutation detection rate is complicated by the large size of the *NIPBL* gene and incomplete characterization of the entire coding region [Gillis et al., 2004]. The development of an auxiliary test, such as screening for PSCS, could help with supporting a diagnosis in individuals with CdLS in whom an *NIPBL* mutation has not been identified, or in whom testing was not performed, as *NIPBL* mutational analysis is not clinically available as of the writing of this manuscript. Further work is needed to determine whether PSCS can be induced in all individuals with CdLS by altering culture or pellet preparation conditions (i.e., tonicity of media, temperature) without affecting the rate of this phenomenon in unaffected individuals.

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Fig. 1.
Full face and profile of a child with typical facial features of Cornelia de Lange syndrome.
Note ptosis, synophrys, long eyelashes, depressed nasal bridge with upturned nasal tip,
posterior-rotated ears, thin upper lip with down-turned corners and a small chin.

Fig. 2.

Metaphase spreads in individuals with CdLS and unaffected controls. **A:** Control metaphase spread stained with giemsa. **B:** Control C-stained metaphase. **C–E:** Metaphase spread from three individuals with CdLS stained with giemsa demonstrating PSCS. Note separated sister chromatids and centromeres present in practically all sister chromatids. **F–H:** C-stained metaphases from individuals with CdLS demonstrating the premature division and separation of the centromeres in the majority of sister chromatids. **I:** A metaphase from an individual with CdLS demonstrating apparent chromatid breaks (arrowheads).

TABLE I
Observed Instances of PSCS in Metaphase Spreads From CdLS Probands and Controls

CdLS probands (N = 90)				
NIPBL mutation positive (N = 40)		NIPBL mutation negative (N = 50)		Controls (N = 90)
PSCS positive	PSCS negative	PSCS positive	PSCS negative	
16/40 (40%)	24/40 (60%)	21/50 (42%)*	29/50 (58%)	8/90 (9%)
Total PSCS positive: 37/90 (41%)*				Total PSCS positive: 8/90 (9%)*
				82/90 (91%)

* Chi-square test demonstrates a *P*-value = 0.0000003.

TABLE II
 Characteristics of PSCS-Positive Individuals With CdLS

Proband #	PSCS (%)	Male(M)/Female(F)	Mutation
1	6	M	p.A1246G
2	6	F	Negative
3	4	M	g.3057–3060delTAGA
4	6	M	p.R1723X
5	4	F	Negative
6	6	F	Negative
7	4	F	Negative
8	2	M	Negative
9	4	F	Negative
10	6	F	Negative
11	4	F	g.961delA
12	2	F	g.1669–1670insC
13	4	M	p.R2298H
14	2	F	Negative
15	2	F	Negative
16	6	M	p.R832X
17	2	F	Negative
18	2	F	Negative
19	4	F	g.2479–2480delAG
20	2	M	Negative
21	2	M	Negative
22	2	M	p.R2298H
23	4	M	g.6763 + 5 G > T
24	6	M	Negative
25	4	F	g.4069–4070insG
26	4	M	p.G2381A
27	10	F	Negative
28	8	M	Negative
29	2	F	Negative
30	6	F	g.7825–7826insG
31	6	F	Negative
32	6	F	p.S1459X
33	6	F	Negative
34	4	F	Negative
35	2	F	Negative
36	4	M	g.5721–5725delTGAAA
37	2	M	p.R479X