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Authors

Calof, Anne L
Kawauchi, Shimako
Santos, Rosaysela
et al.

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Interestingly, protein abundance differences were also detected between patients affected by different *SMC1A* mutations as well as between patients with SMC1A and *SMC3* mutations. Among the visualized variations, several protein spots were identified applying MALDI-TOF MS and ESI-Ion trap MS/MS.

In conclusion, our data could provide a precious tool in understanding biomolecular bases of CdLS pathogenesis.

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Insights Into Cornelia de Lange Syndrome From the *Nipbl*-Mutant Mouse

Anne L. Calof[1,2,3,4,5], Shimako Kawauchi[1,2,3,4], Rosaysela Santos[1,3,4], Martha E. Lopez-Burks[2,3], Michelle P. Hoang[2,3], Leonard M. Kitzes[1,4], Taotao Lao[6], Mark S. Lechner[6], Benedikt Hallgrimsson[7], Jeremy A. Daniel[8], André Nussenzweig[8], Arthur D. Lander[2,3,5]

[1]Department of Anatomy and Neurobiology, [2]Department of Developmental and Cell Biology, [3]Developmental Biology Center, [4]Center for Hearing Research, [5]Center for Complex Biological Systems, University of California, Irvine, California; [6]Department of Bioscience and Biotechnology, Drexel University, Philadelphia, Pennsylvania; [7]Department of Cell Biology and Anatomy, University of Calgary, Calgary, Alberta, Canada; [8]Experimental Immunology Branch, NCI/NIH, Bethesda, Maryland

More than half of all cases of Cornelia de Lange syndrome (CdLS), a multi-organ system birth defects syndrome, are caused by heterozygous mutations in *Nipped-B-like* (*NIPBL*). The orthologue of *Drosophila Nipped-B* and yeast Scc2, *NIPBL* encodes a cohesin-regulatory protein implicated in sister chromatid cohesion, DNA repair and, recently, transcriptional regulation. As a step toward determining how partial loss of NIPBL function gives rise to pervasive birth defects, and in order to develop a model system in which to elucidate mechanisms of NIPBL action, we developed a mouse model for CdLS, using mice heterozygous for a gene-trap mutation (*Nipbl*^{564}) that is predicted to produce a transcript that terminates before the first coding exon. Over 1,000 progeny of the original chimeras and their offspring, spanning five generations of out-crossing onto an outbred background, have now been studied. Among the phenotypes commonly observed are marked pre- and postnatal growth retardation, lean body habitus, cardiac septal defects, delayed ossification, craniofacial dysmorphia (microbrachycephaly, upturned nose), corneal scarring, hearing loss, mild midline cerebellar hypoplasia, seizures, and a variety of abnormal behaviors. Nearly 80% of *Nipbl*$^{564/+}$ mice die prior to weaning. Overall, the phenotypic overlap with CdLS is high, but not total (e.g., limb truncations are not observed in the mice). Analysis of *Nipbl*$^{564/+}$ cells revealed no evidence for elevated precocious sister chromatid separation, implying that the degree of *Nipbl* loss is insufficient to compromise cohesion. Interestingly, in all *Nipbl*$^{564/+}$ cells and tissues examined, *Nipbl* transcript levels were reduced only 25–35%, suggesting upregulation of the wild-type allele. The results of gene expression profiling of E15.5 embryo fibroblasts (MEFs) and E13.5 brain revealed transcriptional changes that were numerous but usually small, only rarely exceeding increases or decreases of greater than 50%. In MEFs, a significant number of the affected transcripts encoded proteins involved in the control of adipocyte differentiation. When assayed, mutant MEFs displayed a significant defect in spontaneous, in vitro adipogenic differentiation, suggesting that these transcriptional changes may play a role in the substantial reduction in body fat observed in *Nipbl*$^{564/+}$ mice. Other transcriptional alterations in *Nipbl*$^{564/+}$ mice involved known locus control regions (beta-globin, H19) and gene clusters. In one such case (the protocadherin-beta cluster), we observed graded changes in expression depending on gene location within the cluster. Overall the data suggest that small changes in the expression of multiple genes underlie that phenotypic features of CdLS, and support a role for Nipbl in regulating long-range chromosomal interactions.

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Pursuit of Additional 'Cohesinopathy' Loci Involved in Human Developmental Disorders

Matthew A. Deardorff[1], Elizabeth Loy[1], Maninder Kaur[1], Micah Berman[1], Laura Conlin[1], Xiaowu Gai[2], Juan Perin[2], Tamim Shaikh[1], Hakon Hakonarson[3], Laird Jackson[4], Ian Krantz[1]

[1]Division of Human Genetics, [2]Division of Bioinformatics, [3]Center for Applied Genomics, Children's Hospital of Philadelphia, PA; [4]Drexel School of Medicine, Philadelphia, Pennsylvania

The Cornelia de Lange syndrome (CdLS) or Brachmann-de Lange syndrome (BDLS) (OMIM 122470) is a multisystem developmental disorder characterized by facial dysmorphia, hirsutism, growth and cognitive retardation, gastrointestinal abnormalities and limb deficiencies. To date, we have identified mutations in approximately 65% of patients with CdLS. These mutations involve the genes *NIPBL*, *SMC1A* and *SMC3*, all of which are involved in sister chromatid cohesion. We have yet to elucidate a cause of CdLS in 35% of patients with typical features of CdLS and nearly 80% of patients with variant features of CdLS. To facilitate identification of genes that may cause CdLS or these variant phenotypes, we have used genome-wide CNV analysis. To date, 269 individuals submitted to our study for whom causative mutations had not been previously identified were analyzed for potentially pathogenic copy number variations using Illumina HapMap550K SNP arrays. Analysis to the level of two consecutive abnormal SNPs has revealed 8,932 potential variants ranging from 2 bp to 20 Mb. Of these CNVs, 5,191 were five SNPs or less.

We have identified deletions in five patients that include *NIPBL* and were validated using MLPA. There were no deletions that included *SMC1A* and *SMC3*, consistent with previous findings of only missense mutations in these genes. We have identified a number of large chromosomal abnormalities in individuals with phenotypic overlap with CdLS, but who do not meet full clinical criteria.

We are hypothesizing that these overlapping clinical features may be similar to CdLS due to the involvement of additional genes involved in the pathogenesis of the "Cohesinopathies."

In our cohort, we have identified two patients with overlapping de novo deletions on 19p13 and two additional patients with overlapping de novo duplications on 19q13. We have identified six additional patients with overlapping abnormalities from other sources and are working to collect their detailed clinical and cytogenetic data. We will present our findings and speculate on the underlying basis of these clinical features and how they may be related to Cohesin function during human development.