Title
Cornelia de Lange syndrome: Further delineation of phenotype, cohesin biology and educational focus, 5th Biennial Scientific and Educational Symposium abstracts

Permalink
https://escholarship.org/uc/item/038197pn

Journal
American Journal of Medical Genetics, Part A, 164(6)

ISSN
1552-4825

Authors
Kline, AD
Calof, AL
Schaaf, CA
et al.

Publication Date
2014

DOI
10.1002/ajmg.a.36417

License
CC BY 4.0

Peer reviewed
Cornelia de Lange syndrome (CdLS) is the prototype for the cohesinopathy disorders that have mutations in genes associated with the cohesin subunit in all cells. Roberts syndrome is the next most common cohesinopathy. In addition to the developmental implications of cohesin biology, there is much translational and basic research, with progress towards potential treatment for these conditions. Clinically, there are many issues in CdLS faced by the individual, parents and caretakers, professionals, and schools. The following abstracts are presentations from the 5th Cornelia de Lange Syndrome Scientific and Educational Symposium on June 20–21, 2012, in conjunction with the Cornelia de Lange Syndrome Foundation National Meeting, Lincolnshire, IL. The research committee of the CdLS Foundation organizes the meeting, reviews and accepts abstracts and subsequently disseminates the information to the families. In addition to the basic science and clinical discussions, there were educationally-focused
talks related to practical aspects of management at home and in school. AMA CME credits were provided by Greater Baltimore Medical Center, Baltimore, MD. © 2014 Wiley Periodicals, Inc.

Key words: de Lange syndrome; CdLS; cohesins; intellectual disability; Roberts syndrome; mice; zebrafish; drosophila

ABSTRACTS

Understanding the origins of structural birth defects in CdLS using zebrafish and mouse models of Nipbl deficiency

Anne L. Calof,1,2,3 Akihiko Muto,2,3 Rosayse Santos, 1,3 Shimako Kawauchi, 1,3 Martha Lopez-Burks,2,3 Thomas Schilling,2,3 and Arthur D. Lander2,3

Departments of 1Anatomy & Neurobiology, 2Developmental & Cell Biology, and the 3Center for Complex Biological Systems, University of California, Irvine, California

Most cases of Cornelia de Lange syndrome (CdLS) are caused by haploinsufficiency for Nipped-B-like (NIPBL), which encodes a highly-conserved protein implicated in loading cohesin onto chromosomes. Importantly, recent studies show major roles for cohesin and Nipbl in transcriptional regulation of many genes. Data on cell lines from human patients, as well as animal models of Nipbl deficiency, raise the intriguing possibility that severe developmental defects in CdLS result from the collective action of many otherwise innocuous small changes in gene expression. To gain insights into the combinatorial origins of birth defects in CdLS, as well as the origins of similar non-syndromic birth defects in the human population at large, we are studying developmental defects in mouse and zebrafish models of Nipbl deficiency.

Zebrafish have two nipbl genes, and morpholin (MO) knockdown of these produces a spectrum of specific heart and gut/visceral organ defects with strong parallels to those seen in CdLS. Mo knockdown of nipbl levels alters expression of many genes as early as gastrulation, particularly genes involved in endodermal differentiation and left–right (L–R) patterning. In experiments in which levels of these genes are altered experimentally, we find evidence that both additive and synergistic interactions among them result in developmental defects, supporting the idea that combinatorial changes in expression of multiple genes underlie structural birth defects in CdLS.

Heart defects are common in Nipbl-deficient zebrafish and mice, just as they are in human individuals with CdLS. Approximately 50% of Nipbl+/− mice have major defects in formation of the atrial septum (ASD) at late gestation. Our recent studies show that structural alterations in the hearts of Nipbl+/− mice are observed as early as Day 10.5 of gestation, before septation begins. As in zebrafish, hearts of Nipbl+/− mouse embryos show alterations in expression of genes with known roles in L–R patterning and other aspects of heart development. These observations suggest that heart defects in Nipbl-deficient mice (and in CdLS) result from abnormalities very early in development, consistent with findings in nipbl-deficient zebrafish.

To facilitate investigation of early developmental abnormalities, we have developed new alleles of mouse Nipbl based on a “conditional/invertible” (FLEX) gene-trap strategy. Such alleles allow us to restore or reduce Nipbl function in a tissue and/or time-specific manner. Results of such studies, and their implications for understanding the etiology of defects in the heart as well as other tissues/structures in individuals with CdLS, will be discussed (see also abstract by R. Santos et al., this meeting). Supported by NIH grant P01-HD052860.

Conditional-Invertible Genetic Strategy to Understand the Role of NIPBL Deficiency in the Etiology of Cardiac Defects in CdLS

Rosayse Santos, 1,3 Shimako Kawauchi, 1,3 Martha Lopez-Burks, 2,3 Akihiko Muto, 2,3 Mona Yazdi, 1 Salvador Deniz, 1 Samir Qurashi, 1 Thomas Schilling, 2,3 Arthur D. Lander, 2,3 and Anne L. Calof1,2,3

Departments of 1Anatomy & Neurobiology, 2Developmental & Cell Biology, and 3The Center for Complex Biological Systems, University of California, Irvine, California

The most common cause of Cornelia de Lange syndrome (CdLS) is haploinsufficiency for NIPBL, a gene that encodes a highly-conserved protein with roles in loading cohesin onto chromosomes and transcriptional regulation. Congenital cardiac defects are common in CdLS, as well as in many other birth defects syndromes and in the population as a whole. To better understand the origins of heart defects in CdLS, as well as to gain insight into the origins of non-syndromic heart defects, we are developing mouse models of Nipbl deficiency. Nipbl+/− mice, which recapitulate many CdLS phenotypes, show developmental defects in ventricular septation (VSD) and atrial septation (ASD). Importantly, structural abnormalities and alterations in expression of genes with known roles in both left-right patterning and early heart development are also found. Interestingly, studies of Nipbl-deficient zebrafish—which exhibit specific defects in heart and visceral organ development at high frequency—indicate that early changes in expression of genes involved in endodermal specification (e.g., sox17) and left-right patterning are important in the etiology of heart defects in this model of CdLS. Such results suggest that heart defects in CdLS may result from abnormalities that occur very early in development.

To gain insight into the developmental timing and tissue site(s) of origin of heart defects, we have developed a new Nipbl allelic series in the mouse, based on flip-excision (FLEX) “conditional/invertible” gene-trap strategy. Such alleles have been shown to toggle from mutant, to wildtype, and back to mutant gene

How to Cite this Article:
conformations by sequential introduction of Cre and/or Flp recombinases in vitro. We have generated mice in which one Nipbl allele contains a FlEx gene-trap cassette, and will discuss our data demonstrating that the Nipbl allele can be toggled between mutant and wildtype conformations in vitro and in vivo. Currently, we are using the Nipbl-FlEx allelic series to generate mouse embryos in which Nipbl is inactivated in cardiac mesoderm and/or endoderm during early development, to examine the cellular origins of cardiac developmental defects in CdLS. Experiments are also underway to determine whether restoration of Nipbl to normal levels in specific tissues during development can rescue heart defects in Nipbl-deficient mice. Ultimately, the goal of these studies is to understand how Nipbl deficiency results in structural and genetic changes that underlie developmental heart defects in CdLS. Supported by NIH grants P01-HD052860 and HD052860-03S1-01. RS is the recipient of a President’s Dissertation Year Fellowship from UCI.

Premature Aging and Telomeres in Cornelia de Lange Syndrome

Antonie D. Kline,1 Harold Riethman,2 Krithika Ravichandran,2 Maninder Kaur,3 Amy Kimball,1 and Ian D. Krantz3

1Harvey Institute for Human Genetics, Greater Baltimore Medical Center, Baltimore, Maryland; 2The Wistar Institute, Philadelphia, Pennsylvania; 3Division of Human Genetics and Molecular Biology, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania

Evaluations of multiple individuals with Cornelia de Lange syndrome (CdLS) through an aging clinic, and at regional and national meetings, have shown evidence for premature aging, with some details previously reported. These findings have included faster changes seen in facial features than expected for age with wrinkles and sagging of skin, development of prematurely gray hair, Barrett’s esophagus and cholecystitis occurring at a younger age than expected, prostatic enlargement by the early 40s, early development of osteoporosis, and development of impaired corneal reflex with some evidence for neuropathy and autonomic dysfunction. There have been no increased incidence of malignancy, no significant cardiac involvement or increased risk for hypertension, and the prevalence of diabetes is lower than expected. There is a decline in adaptive skills and increase in neuropsychiatric symptoms after adolescence.

The genes that are involved in CdLS code for cohesin, a protein complex involved in the mitotic relationship of sister chromatids, as well as enhancer-promoter interactions, DNA damage repair, and other embryologic functions. Premature aging has been associated with abnormalities in genome stability, telomere length, integrity and stability, DNA repair, and oxidative stress. In order to pursue the etiology of the premature aging seen in CdLS, we have first undertaken evaluation of telomeres in individuals of different ages. Cohesin’s role in maintenance of telomeres has been unknown, although cohesion at the telomeres is known to play a crucial role in chromosome structure and genomic stability. Telomere characteristics from 11 lymphoblast and fibroblast cell lines from individuals with CdLS with mutations in one of the genes associated with cohesin were compared to six age-matched control cell lines without CdLS. Both older cell lines and more fresh cell lines were evaluated. The telomere repeat lengths were found to be similar to those of controls, with those from blood slightly longer than from skin in both study and control cells. A telomere stability assay, in which the lengths of individual telomeres are compared using a PCR-based assay, showed that there is no difference in the relative fraction of short telomeres in CdLS compared to the controls. Thus, there is no evidence that there are changes in the telomeres in CdLS with aging, including DNA breakage or damage in the telomeres, or shortening of the telomeres. We plan to assay DNA repair in fibroblast cells as compared to controls as the next step in the evaluation of premature aging.

Co-regulation of Developmental Control Genes by Cohesin and Polycomb Group Silencing Proteins

Cheri A. Schaaf, Ziva Misulovin, Maria Gauze, Audrey Watson, and Dale Dorsett

Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, Saint Louis, Missouri

The cohesin protein complex, and the Nipped-B protein required for cohesin to bind to chromosomes, regulate gene transcription. We hypothesize that changes in gene expression underlie the developmental deficits in Cornelia de Lange syndrome (CdLS) caused by heterozygous mutations affecting human cohesin subunits or the human Nipped-B homolog, Nipped-B-Like. Using Drosophila as a model organism, we find that in a cell-type specific manner, some developmental genes bound by cohesin and Nipped-B also bind Polycomb group (PcG) silencing proteins. Strikingly, relative to most cohesin-binding genes, those co-targeted by PcG proteins are exceptionally sensitive to cohesin dosage, and we hypothesize that such genes are likely to change the most in expression in CdLS, where the reductions in cohesin or Nipped-B activity are modest. In contrast to genes that bind PcG proteins without cohesin, these genes are not silenced, but expressed at low to intermediate levels. They increase dramatically in expression upon depletion of cohesin or PcG proteins, but co-depletion does not synergistically increase transcript levels. At some of these genes, cohesin and PcG proteins inhibit binding of the Notch transcriptional activator and alter RNA polymerase activity. We propose that the combined action of cohesin and PcG proteins establishes an intermediate level of gene expression that is critical to achieve the correct cell identity and developmental fate. Supported by NIH grants GM055683 and HD052860.

Using Patient-Derived iPS Cells to Elucidate the Molecular Mechanisms Underlying Cornelia de Lange Syndrome (CdLS)

Dongbin Xu,1 Jason Mills,2 Deborah French,2 Paul Gadue,2 Aaron Dickinson,1 Manindar Kaur,1 and Ian Krantz1,3

1Division of Human Genetics, The Children’s Hospital of Philadelphia, Pennsylvania; 2EHC Core Facility, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania; 3The Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania

Probands with Cornelia de Lange syndrome (CdLS) display various congenital differences such as cognitive and behavioral impairment, growth delays and multiple structural variations. Approximately 65% of CdLS probands have an identifiable mutation in cohesin or cohesin-related genes such as SMC1A, SMC3, NIPBL, and HDAC8. Mutations in these genes seen in CdLS result in disruption of the cohesin complex’s ability to regulate dynamic gene expression, the likely underlying cause of the specific clinical
features seen in CdLS, rather than its canonical role in regulating chromosome segregation. The limitations in studying gene regulation in developing human embryos have prohibited exploring the mechanism of CdLS in a fundamental developmental-specific manner in humans. To address this problem, we have generated a series of induced pluripotent stem cell (iPSC) lines from fibroblast cell lines derived from CdLS probands who have mutations in NIPBL or HDAC8. A set of control iPSC lines were generated in parallel from fibroblast cell lines derived from a healthy control of matched age, race, and gender, as well as from a proband with Roberts syndrome (caused by mutations in ESCO2, a critical gene required for cohesion establishment). In addition to morphology and growth features characteristic of stem-cells, these iPSCs highly express pluripotent genes such as REX1, ABCG2, and DNMT3B, indicating their fully reprogrammed state. Teratomas generated from these iPSCs are under analysis to further validate the pluripotency of these iPSCs. Differentiation of these iPSCs into neuronal cells and cardiomyocytes is ongoing. These differentiation assays will provide invaluable reagents for studying the basic molecular mechanism of CdLS in a cellular model of human cell and tissue-specific development. These studies are anticipated to lead to the identification of downstream “effector” target genes of cohesin action that may be critical to the development of specific tissues and will themselves be candidate disease genes for the isolated structural and cognitive differences seen in constellation in CdLS.

**Cohesin Binding and Gene Expression**

Kyoko Yokomori,1 Richard Chien,1 Daniel A. Newkirk,1,2 Yen-Yun Chen,1 Weihsia Zeng,1 Jacob Biesinger,2 Neil Infante,2 Shimako Kawauchi,3 Rosaysela Santos,3 Anne L. Calof,3 Arthur D. Lander,4 and Xiaohui Xie2

1Department of Biological Chemistry, 2Department of Computer Science, 3Department of Anatomy & Neurobiology, School of Medicine, 4Department of Developmental & Cell Biology, School of Biological Sciences, University of California, Irvine, California

Cohesin is a multiprotein complex required for sister chromatid cohesion and proper segregation of chromosomes in mitosis, but is also involved in gene regulation. Cornelia de Lange syndrome (CdLS) is a human developmental disorder most frequently linked to mutations in the NIPBL gene, an essential loading factor of cohesin, while a smaller proportion of cases result from mutations in cohesin subunits. Haploinsufficiency for NIPBL is associated with the most clinically severe cases of CdLS and is characterized by global alterations of gene expression, not only in human patients, but also in animal models. These observations suggest that the gene regulatory function of cohesin is compromised in conditions of NIPBL deficiency. However, the effect of NIPBL deficiency on cohesin is not well understood, and the identities of cohesin target genes relevant to CdLS phenotypes are largely unknown. Recent studies suggest the existence of two distinct classes of cohesin binding sites, one overlapping with CTCF and which recruits cohesin, the other acting without CTCF. Nipbl has been suggested to be particularly important for the latter. Using mouse embryonic fibroblasts (MEFs) derived from Nipbl+/− mice that closely recapitulate CdLS phenotypes, we performed a molecular analysis to determine how Nipbl deficiency affects cohesin binding and gene expression genome-wide. Our results provide important insights into the effect of Nipbl deficiency on the regulation of cohesin target genes that contribute to CdLS. This work was supported by NIH grants R21 HD062951 (KY) and P01-HD052860 (ALC, ADL).

**Nutrition in Cornelia de Lange Syndrome**

Loretta Harvey,1 Alicia Mangiaficoa,2 Deirdre Summa,3 and Antonie D. Kline4

1University of Maryland Medical Center, Baltimore, Maryland; 2Eastern Connecticut State University, Willimantic, Connecticut; 3Cornelia de Lange Syndrome Foundation, Avon, Connecticut; 4Harvey Institute for Human Genetics, Greater Baltimore Medical Center, Baltimore, Maryland

Nutrition plays a vital role in the growth and development of children. For those affected with Cornelia de Lange syndrome (CdLS), obtaining proper nutrition may be an obstacle due to the challenges associated with the syndrome. These include feeding and/or swallowing dysfunction, gastroesophageal reflux, bowel motility issues, and neurodevelopmental problems. Our hypothesis was that there are dietary and nutritional issues in CdLS that are
different from unaffected children and that these issues are likely different at different ages. We felt that answers to this topic could help with intervention and management. Following IRB approval, an on-line nutritional survey was completed by parents or caretakers of individuals with CdLS who were previously seen through the Multidisciplinary Aging Clinic for CdLS at the Greater Baltimore Medical Center or accessed through the Cornelia de Lange Syndrome Foundation. The study questions addressed growth concerns, feeding issues, food allergies/intolerances and gastrointestinal issues. Two hundred and two parents or caregivers completed the on-line survey in the summer of 2011. Results of the survey will be presented. This study proves our hypothesis to be correct, and indicates the need to prospectively address issues related to nutrition in this population. Further studies are indicated.

Dosage-Sensitive Regulation of Drosophila Growth, Metabolism and Development by Nipped-B

Maria Gauze, Cheri A Schaaf, Caleb Ford, Carolyn Albert, David Ford, and Dale Dorsett

Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, Saint Louis, Missouri

Individuals with Cornelia de Lange syndrome display slow growth and multiple developmental deficits. We are investigating the etiology of CdLS using Drosophila as a model organism. We originally isolated Drosophila Nipped-B mutations in a genetic screen for factors that regulate two homeobox genes that control development, cut and Ultrabithorax. Using genome-wide analysis, we find that in addition to cut and Ultrabithorax, Nipped-B is required for both cut and Ultrabithorax. Using genome-wide analysis, we find that in addition to cut and Ultrabithorax, Nipped-B mutants alter the expression of hundreds of genes in developing wings and brain, similar to expression changes that occur in cells from individuals with CdLS, Nipbl mutant mice, and zebrafish treated with anti-Nipbl morpholinos. One of the genes affected in all organisms is myc, a key regulator of protein synthesis, cell division, and growth.

We find that Nipped-B mutants have a growth defect without a developmental delay. They reach defined developmental stages from embryogenesis to adulthood at the same times as genetically-matched wild-type controls, on both rich and restricted diets. On both diets, however, they are 10–15% smaller than wild-type. Increased expression of myc rescues wing size defects.

Relative to controls, Nipped-B mutant adults also show increased ability to survive starvation when grown on rich food, but decreased ability when grown on a restricted diet, suggesting there is dysregulation of energy homeostasis. The altered responses to starvation do not stem from changes in triglyceride levels. Supported by NIH grant P01 HD052860.

Feeding and Related Issues in Individuals With Cornelia de Lange Syndrome

Cheri S. Carrico

Elmhurst College, Elmhurst, Illinois

Cornelia de Lange syndrome (CdLS), also known as Brachmann-de Lange syndrome, is a congenital syndrome in which affected individuals exhibit atypical facial, physical, and developmental characteristics that may be associated with feeding difficulties. Micrognathia, a weak bite, and cleft palate may impact chewing and swallowing. Delayed physical growth, gastroesophageal reflux disease, and possible failure to thrive may be associated with poor nutritional intake. Reflux, tube feeding, autistic tendencies, and sensory issues are linked with food aversions. Speech-language delays, apraxia of speech, developmental delays, and self-injurious behaviors also are common.

Because feeding issues are prevalent among individuals with CdLS, specific feeding difficulties of 59 affected individuals, age 3 months through 36 years, were investigated, based on observations of feeding behaviors and information reported by caregivers. Significant findings included choking, coughing, gagging, vomiting, and spitting food out at meal times; refusal to feed orally; oral defensiveness; and a history of tube feeding and gastroesophageal reflux disease (GERD). Additional feeding concerns included difficulty sucking, swallowing, chewing, and biting; consumption of only small bites of food; lack of transition to solid foods; lack of transition to cup drinking; selective eating habits; messy feeding habits; and slow feeding.

Health concerns that may be associated with feeding difficulties included colds, ear infections, pneumonia, sinusitis, fevers, sepsis, allergies, and lactose intolerance. Primary feeding concerns reported by caregivers included transitioning to oral feeding, promoting eating of solid foods, promoting eating of foods of various textures, encouraging better overall eating habits, and developing appropriate feeding techniques.

Techniques to improve oral feeding ability vary with the needs of the individual. Oral stimulation should be provided as early as possible, including in children who are tube fed. Among individuals who cannot tolerate oral presentation of food, non-nutritive, oral stimulation should be provided, using safe items of various textures and temperatures. When it is medically safe, taste stimulation may be provided by adding tiny amounts of liquids or semisolids to pacifiers, fingers, toys, and so forth. When able to safely feed orally, typically semi-solids are introduced first, followed by solids and liquids. If an individual cannot safely swallow without choking, coughing, gagging, retching, or aspirating, it may be helpful to adjust the food’s thickness. Individuals who cannot safely manage liquids sometimes can safely manage soft solids. Initially introduce only one texture until the individual is comfortable and feeding safely. Next, the texture may be modified by varying the consistency. Many individuals express an initial preference for bland foods and later move toward foods with stronger flavors; however, some individuals prefer spicier foods first and gradually will choose foods that are blander. All feeding should occur in a comfortable, friendly environment. Before attempting feeding therapy, the individual must be medically safe to feed orally, and food allergies should be ruled out.

Modulation of Scc2/delangin Chromatin Association by Phosphorylation

Julie Woodman and Paul Megee

Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado

Although a clinical description of Cornelia de Lange syndrome (CdLS) has been developed, much less is known regarding its molecular origins. CdLS patients exhibit increased sensitivity to DNA damage, some degree of precocious sister chromatid separation, and defects in developmental gene regulation, all processes mediated by the evolutionarily conserved cohesin complex.
genetic analyses reveal that a subset of CdLS mutations maps to genes encoding Smc1 or Smc3, two subunits of cohesin, the majority of CdLS patients exhibit genetic mutations in NIPBL/delangin, one of the two subunits of cohesin’s chromatin deposition factor. When cohesin is unable to associate with its specific chromosomal locations, genes that must be precisely regulated during development lose their ability to coordinate expression. It is therefore proposed that proper cohesin deposition onto chromatin is required to avoid the severe developmental delays observed in CdLS patients. Key to understanding the role of the cohesin deposition complex in gene regulation is to elucidate precisely how NIPBL/delangin functions to properly target cohesin to chromosomes. In this study, we investigated how Scc2, the budding yeast NIPBL ortholog, is first recruited to, and subsequently associated with, its chromosomal locations. The data indicate that Scc2 is phosphorylated and that its chromatin association increases as cells progress through the cell cycle. Interestingly, the aforementioned increase in chromatin binding activity occurs after the essential requirement for cohesin deposition during DNA replication has been established, suggesting that the deposition complex may have as yet unidentified post-replication function(s). Scc2 phosphorylation also appears to play a critical role in mediating an interaction with its binding partner, Scc4, as well as with the cohesin subunit, Mcd1. Collectively, these results suggest a mechanism of cohesin deposition that is mediated by the cell cycle regulated phosphorylation of Scc2.

**Protein Translation and Roberts Syndrome**

Baoshan Xu, Tania Bose, Kenny Lee, Shuai Lu, Bethany Harris, Chris Seidel, and Jennifer L. Gerton

Stowers Institute for Medical Research

Roberts syndrome (RBS) is a human developmental disorder characterized by craniofacial and limb defects. RBS is caused by mutations in ESCO2, a cohesin acetyltransferase. We have explored the idea that reduced ribosome function contributes to the development of this disease. We find that the production of ribosomal RNA is reduced in a human RBS fibroblast line. Since these RNAs limit ribosome biogenesis, we tested whether protein synthesis was reduced and whether there were fewer actively translating ribosomes. By both measures, protein translation is impaired. One key pathway controlling translation is mTOR (mammalian target of rapamycin). We find that mTOR signaling is inhibited in human RBS fibroblasts through S6K1 and 4E-BP1, both downstream effectors of mTOR, and this correlates with an elevation in p53. We have tested the effect of inhibiting p53 or stimulating mTOR in human RBS fibroblasts. Both treatments partially rescue the defects associated with ESCO2 mutation. We will discuss whether RBS and other cohesinopathies might be "ribosomopathies," defined as diseases associated with defects in ribosome function.

**Cohesin Complex Regulator ESCO2 Interacts Directly With the Replisome Progression Complex Members Timeless and TIPIN**

Hugo Vega,1,2 Miriam Gordillo,2,3 Hee Soul-Rho,4,5 Heng Zhu,4,5,6,7 and Ethylin Wang Jabs2,8

1Instituto de Genetica, Universidad Nacional de Colombia, Bogota, Colombia; 2Department of Genetic and Genomic Sciences, Mount Sinai School of Medicine, New York, New York; 3Department of Surgery, Weill Cornell Medical College, Cornell University, New York, New York; 4The Center for High-Throughput Biology, Departments of 5Molecular Biology and Genetics, 6Pharmacology and Molecular Sciences, 7The Sidney Kimmel Comprehensive Cancer Center, and 8Departments of Pediatrics, Medicine, and Surgery Johns Hopkins University School of Medicine, Baltimore, Maryland

ESCO2 mutations cause Roberts syndrome (RBS). ESCO2 is member of the Eco1 family of acetyltransferases that is central to the establishment of sister chromatid cohesion, a process intimately linked to the passage of the replisome. Eco1 is aided during cohesion establishment by a number of non-essential factors associated to the replisome. However little is known about how they link replication and cohesion. Using a human protein chip, we searched for interacting proteins of the ECO1 human paralogs ESCO2 and ESCO1. We found that the amino end of ESCO2 and ESCO1 physically interact with the carboxy end of Timeless, a member of the replisome progression complex (RPC). ESCO2 exists in two different complexes at the replication fork one with PCNA and other with Timeless and Tipin. Timeless-TIPIN-ESCO2-ESCO1 complex appears to have a major role on sister chromatid cohesion. RBS cells, deficient in ESCO2 activity, showed reduced levels of Timeless, abnormal electrophoretic mobility of Tipin, and increased amount of PCNA on the chromatin. ESCO2 is interacting with members of the RPC and in this way is positioned to regulate establishment of cohesion and progression of the replication fork.

**Ocular Manifestations of CdLS: Questions Waiting to be Answered**

Alex V. Levin

Wills Eye Institute, Thomas Jefferson University, Philadelphia, Pennsylvania

The most common ocular manifestations of CdLS, myopia, blepharitis, and ptosis, offer research opportunities to better understand the nature of these conditions. What is it about this cohesinopathy syndrome that causes these specific ocular manifestations to occur? There are multiple genetic loci that have been identified for myopia but less is known about the genetic manifestations of ptosis, and even less regarding blepharitis. Congenital myopia, a disorder of ocular growth regulation, is a complex disease which in the case of CdLS, occurs without the environmental influences sometimes attributed to the acquired forms of this disorder such as high reading levels. Ptosis, is due to a congenital underdevelopment of the levator muscle. Like myopia, ptosis is found in many other syndromic disorders. Blepharitis is largely considered a mechanical dysfunction of the eyelid meibomian glands. Although its prevalence is increased in a few genetic syndromes (e.g., trisomy 21), it is not generally considered a genetic disorder. Mouse models of CdLS have shown corneal surface disease, which may in part be related to lid dysfunction. Likewise, mild microcornea is a manifestation of CdLS.

This talk will examine the possible genetic pathophysiology of the ocular manifestations of CdLS based on our current knowledge regarding the genetics of the syndrome. The presenter will also offer a potential research agenda to address these questions.

**Genome Wide Localization Study of Acetylated Cohesin in Human**

Masashi Minamino, Masashige Bando, Yuki Katou, Komata Makiko, Ryuichio Nakato, and Katsuhiko Shirahige
Cohesin complex consists of four subunits, Smc1, Smc3, Rad21, and SA1/2, and regulates sister chromatids cohesion as well as gene expression in higher eukaryotes. In human, Esco1 and Esco2 are cohesin-specific acetyltransferases that are required for establishment of sister chromatid cohesion. Sister-chromatid cohesion is established during S phase, and acetylation of SMC3 by ESCO1 and ESCO2 is required for cohesion. We found that Smc3 acetylation in G1 phase depends solely on Esco1, while in S phase, acetylation depends on both Escos. In good agreement with this observation, Esco1 binds to chromosome throughout the cell cycle, whereas Esco2’s localization on chromosome is only detectable in S phase. Our genetic screening for possible interacting factor with Esco1 identified PDS5 as a protein involved in the same pathway for acetylation of Smc3. Interestingly, Esco1 is phosphorylated in mitotic phase by Aurora B kinase, suggesting that Aurora B may inactivate Esco1 by phosphorylation and negatively regulate acetylation of Smc3.

Using monoclonal antibody that specifically detect acetylated from of Smc3, we carried out ChIP-seq analyses of Smc3-ac and Rad21 in untreated, esco1 depleted, and esco2 depleted background. The results clearly showed that Smc3-ac tends to accumulate at 3’ side of ORF, and this accumulation depends on transcriptional activity of the ORF. Depletion of esco1 results in 60% loss of Smc3-ac localization sites whereas deletion of esco2 results in 20% loss. Interestingly, when we analyzed localization of Smc3-ac in repetitive sequences, Smc3-ac localization at several classes of centromeric repeat was specifically decreased by depletion of Esco2. Specific role of each Esco will be discussed.

Education and CdLS

Michele Champion,1 Mary Levis,2 Janette Peracchio,3 and Elizabeth Fouts4

1Lawrence Public Schools, Lawrence, Massachusetts, 2Wicomico County Board of Education, Salisbury, Maryland, 3Cornelia de Lange Syndrome Foundation, Avon, Connecticut 4Saint Anselm College, Manchester, New Hampshire

Mary Morse, Ph.D., had a vision for the CdLS Foundation and that vision was providing educational support for professionals, families and caregivers of children and adults with Cornelia de Lange Syndrome. During the many years of Dr. Morse’s service to the foundation, she has provided a wonderful foundation of educational articles, recommendations, and support to our special children. The goal of the Educational Advisory Group (EAG) is to fulfill that vision through providing resources about the unique educational needs of children and adults with CdLS. The EAG is comprised of professionals in the field of education across the United States. The group continues to evolve and grow as we add more specialists in various areas of therapies and education.

One immediate goal of the EAG is to put together an Educational Handbook that can be used by professionals and families in every state. It will be divided into chapters, such as Know Your Rights, IDEA; IEP; Advocacy; Communication; Assessments; Behavior. Currently three chapters are up for review by the Professional Development Committee. Members of the EAG will serve as a resource to families and professionals by answering educational questions that families and professionals may have and offer recommendations to people educating students who have CdLS. A log of questions and recommendations will be maintained to be used as a future resource. Members of the EAG will attend local gatherings and conferences to meet and help families and children with CdLS. Members of the EAG will also share their knowledge by writing fact sheets, articles for Reaching Out and speaking at foundation events. The EAG meets monthly via conference calls and email.

As chairperson of the EAG, and with my committee, we are pleased to be involved in the development of the EAG to address and develop appropriate educational practices and services for the professionals who serve children with CdLS and families of children and adults with CdLS all over the United States.

Contrasts in Communication Development Among the Normal, Autistic, Nonspecific Cognitively Impaired, and Cornelia de Lange Syndrome Populations

Marjorie T. Goodban
Elmhurst College, Elmhurst, Illinois

Background: CdLS is associated with severe deficits in speech and language development. The results of speech and language consultations conducted over the past 27 years with patients attending regional, national, and international CdLS conferences will be presented along with the results of two databases. These results will be used to contrast and compare both speech and language development and disorders found in the following populations: normal, autism spectrum disorder (ASD), nonspecific cognitively impaired, and CdLS.

Methods: Families and their family member with CdLS were seen for consultations at regional, national, and international conferences over a period of 27 years. Two databases were developed from these consultations and analyzed for relationships among speech outcome and other factors. Findings were then compared to communication development and disorders typically found in normal, ASD, and nonspecific cognitively impaired populations.

Results: Important differences in communication development and disorders exist among the normal, nonspecific cognitively impaired, and CdLS populations. These differences include speech apraxia, history of babbling, ASD, unusual results of hearing tests, vocal quality, asynchronous development of language skills, reduced speech intelligibility, structural differences of the oral cavity, cleft palate, differences in the development of syntax, selective mutism, and disparities between expressive language and cognitive abilities.

Discussion: The severity of communication disorders in CdLS tends to more severe than those found in the nonspecific cognitively impaired population and as challenging if not more so than those found in ASD. Parents may need extra support and guidance as they seek treatment for their family member with CdLS.

Working with Individuals With Challenging Behaviors

Julia T. O’Connor
Kennedy Krieger Institute and Johns Hopkins University School of Medicine

Individuals with developmental disabilities are at greater risk for also developing challenging behaviors. Research has shown that having to cope with challenging behaviors is a major stressor for families. Additionally, these behaviors can also be a major stressor...
for those professionals providing ongoing care or services to the individual.

Challenging behaviors include self-injury, aggression, disruption, elopement, and noncompliance (just to name a few). Such behaviors often limit the individual’s ability to access the services necessary to make ongoing gains. As such, individuals often are unable to receive the needed medical care, educational services, or ancillary therapies that are so important for further skill development. Additionally, challenging behaviors often persist unless interventions are implemented. These challenges will be discussed.

In this talk, a model will be reviewed for professionals (medical personnel, teachers, and therapists) to use to approach the care of an individual with autism and/or severe behavioral issues. This model includes the use of a comprehensive medical workup, behavioral assessment to identify potential variables that maintain challenging behaviors and treatment options related to the function of problem behaviors. Case examples will be presented.

**Foundations of Communication: Social Orientation and Joint Attention**

Mary Papan


Well before a child learns to talk, they are attending to and processing the actions of social beings within their environment. It is well established that newborn infants have a strong preference for the human face, and somewhat later, the human voice. Such social orienting seems to be “hard-wired,” that is innate rather than learned. It has also been well established that such social orienting does not occur as robustly in young children with autism. Such a lack of social orienting often gives caregivers one of the first indications that something is wrong with their child.

When a child socially orients toward another, then is able to follow that person’s attention to a third object, they are establishing joint attention. Both are looking at the third object and both know that the other is also looking at that object. Soon a child learns how to also direct the attention of another to a third object, at first simply through eye gaze, then adding a point, then finally through language as well. This process of joint attention, especially joint attention to an interest rather than a want, has also been shown to be lacking in children with autism.

When an infant lacks social orienting, and then fails to establish joint attention skills, they miss out on a huge learning opportunity from which most children learn socialization, communication, and language skills. Such skills are foundational for later learning. With the recognition that these skills are so vital to later development, recent work has focused on ways to develop these skills in children who lack them or are at high risk of poor development in this area, such as children with CdLS.

This presentation will introduce the above concepts to the audience as well as review evidence based programs for building social orienting and joint attention skills in infants and children who show early signs of deficit in these areas.

**Is Cell Fate Sealed by Cohesin? Modeling Mechanisms of Cohesin-Dependent Gene Expression in Zebrafish**

A. O’Neill, B. Kao, J. Marsman, H. More, J. Rhodes, M. Mönich, S. Young, and J. Horsfield

Department of Pathology, University of Otago, Dunedin, New Zealand

Mutations in subunits of the cohesin complex, or its regulators, cause human developmental disorders such as Cornelia de Lange syndrome and Roberts syndrome. Cohesin and CCCTC binding factor (CTCF) have important roles in genome organization and gene expression; cohesin frequently co-locates with CTCF throughout the genome, and can control enhancer-promoter communication to regulate gene expression. Research from our lab using the zebrafish animal model has provided specific examples where a transcriptional role of cohesin links cell proliferation to differentiation.

One of the early examples of cohesin-dependent gene regulation was our finding that cohesin is required for tissue specific transcription of runx1, a gene important for hematopoietic stem cell development. We observed that runx1 activation in a specific hematopoietic cell population in zebrafish embryos—the posterior lateral mesoderm (PLM)—depends on cohesin; however, the mechanism for this regulation was unknown. Our recent data shows that cohesin initiates expression of runx1 in the PLM, while CTCF restricts its expression in the newly emerging cells of the tail bud. We identified two intrinsic runx1 enhancers, and found that cohesin depletion diminished their activity and abrogated binding of RNA Pol II to the runx1 enhancers and promoters. Binding sites within runx1 for cohesin and CTCF also suggest the presence of putative insulator elements that could restrict runx1 transcription in emerging tail bud cells. We propose a model describing how CTCF-dependent chromatin structures might inhibit runx1 expression, while cohesin unlocks the runx1 locus for transcription in hematopoietic cells.

There has been recent interest in the potential for cohesin to participate in steroid hormone signaling. Studies in breast cancer cells indicate that cohesin binds at specific chromosome locations together with estrogen receptor (ER-alpha), in response to estradiol. Recently, we used a transgenic zebrafish model to show that cohesin might directly modulate ER-alpha signaling through estrogen response elements. Cohesin’s role in estrogen signaling in the zebrafish model does not appear to involve CTCF function or chromosome looping. Investigation of the mechanism(s) for cohesin’s role in the estrogen response are ongoing.

**Range of Features in Patients With Cohesin Gene Mutations**

Matthew A. Deardorff

University of Pennsylvania Perelman School of Medicine, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania

Cornelia de Lange syndrome (CdLS) is a dominantly inherited multisystem developmental disorder that includes distinctive facial features, growth retardation, a range of limb defects and variable cognitive delay. Over the past 7 years, we have identified causative mutations for many individuals with CdLS. However, a range of clinical phenotypes exists that overlap with CdLS but don’t fully meet the diagnostic features.

Mutations in the NIPBL gene have been identified in approximately 75% of patients with severe classical CdLS but have been found in a far lower percentage of patients with mild or atypical features. Some of these patients have had mutations identified in the SMC1A or SMC3 genes, which typically present with milder...
structural anomalies, more fullness of the eyebrows, a more prominent nose and significant cognitive impairment.

More recently, we have identified mutations in the RAD21 and HDAC8 genes. Although we have identified only three de novo RAD21 mutations to date, these individuals display a less striking clinical phenotype, most with very mild cognitive impairment. Even within this small group, there is heterogeneity of clinical features, which we feel to be likely due to the nature of the type of RAD21 mutation. The identification of additional individuals will serve to further delineate this subgroup of features.

Mutations in HDAC8 cause clinical features that are quite similar to classical CdLS, caused by NIPBL mutations. However, there is some degree of difference in severity within families as well as between males and females. The majority of this variability is likely due to the degree to which the mutant allele is expressed for this X-linked gene.

I will review the underlying causes and clinical features of these five molecular subgroups and propose reasons why mutations in these different genes may result in varying clinical features.

**Modeling Cornelia de Lange Syndrome (CdLS) With Drosophila melanogaster**

Dongbin Xu,1 Jennifer Li,2 Wenfeng Chen,3 Aaron Bell,4 Richard Tilton,5 Emily Gallant,1 Emily Jones,2 Thomas Jongens,4 and Ian Krantz1,4

1Division of Human Genetics, The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania; 2School of Arts and Sciences, University of Pennsylvania, Philadelphia, Pennsylvania; 3Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania; 4Department of Genetics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania; 5School of Medicine, Temple University, Philadelphia, Pennsylvania

Cornelia de Lange syndrome (CdLS) is a dominant genetically heterogeneous diagnosis characterized by a striking constellation of cognitive impairment, growth delay and birth defects. Approximately 65% of CdLS probands have an identifiable mutation in cohesin structural (SMC1A, SMC3) or regulatory (NIPBL and HDAC8) genes. We, and others, have described a non-canonical role for cohesin as a critical regulator of gene expression, that is most likely the mechanism by which it mediates its phenotypic effect in CdLS. Homozygous mutations of cohesin factors in Drosophila (and other organisms) is embryonically lethal, while heterozygous loss has minimal, if any, reported phenotypic effect. Targeted homozygous mutation of cohesin genes or disruption of cohesin structure has been shown to cause failure of γ-neuron pruning, a post-mitotic event, during Drosophila brain development. It remains unclear if loss of one copy of these cohesin genes, more closely representing the gene dosage in CdLS probands, also causes any structural or functional brain differences in Drosophila. We investigated brain development in Drosophila heterozygous mutants of cohesin structural and regulatory genes and observed γ-neuron pruning defects in mushroom bodies during the pupal stage. γ-neurons failed to be pruned at 20 hr after pupae formation in approximately 30% of the SMC1, Rad21, Rpd3 (Drosophila HDAC8 homologue) and Nipped-B heterozygous mutant pupae. This indicates that heterozygous cohesin mutations do exert an effect on normal brain development in the fly, although the effect is not fully penetrant. Although we have documented normal locomotor activity, in these heterozygous mutant flies, they do display irregular sleep rhythm. Many of these mutants present decreased total sleep time consisting of a larger number of short sleep episodes compared with wild type flies. These flies also tend not to sleep well during dark periods. Preliminary courtship assays demonstrates a deficit in learning and memory function in the heterozygous cohesin-associated mutant flies. Parallel genome-wide expression analysis of the brain of these mutant flies will provide molecular insight into the effects of cohesin genes’ haploinsufficiency on gene regulation. These experiments will be very important in understanding the alterations of the transcriptome in the Drosophila brain and how that translates into the cognitive status resulting from haploinsufficiency. Developmental differences were also observed in the heart and in pattern formation of eye bristles between heterozygous mutant and wild type flies. These and ongoing studies will help to validate if the heterozygous cohesin mutant flies echo the phenotype seen in CdLS probands and can serve as a valuable model to study organ/tissue-specific molecular pathogenesis in CdLS during development and to screen potential therapeutic drugs.

**Insomnia in Cornelia De Lange Syndrome**

Roy Rajan,1 James R. Benke,2 Antonie D. Kline,3 Howard P. Levy,4 Amy Kimball,5 Tiffany L. Mettel,2 Emily F. Boss,2 and Stacey L. Ishman5

1Emory University, Department of Otolaryngology—Head and Neck Surgery, Atlanta, Georgia; 2Department of Otolaryngology—Head and Neck Surgery, Johns Hopkins University School of Medicine; 3Greater Baltimore Medical Center, Harvey Institute of Human Genetics, Baltimore, Maryland; and 4Department of Medicine and McKusick-Nathans Institute of Genetic Medicine, Baltimore, Maryland

**Objective:** Up to 55% of patients with Cornelia de Lange Syndrome (CdLS) experience sleep disturbance. Prior evaluation of children without CdLS with similar intellectual disability and self-injurious behavior suggests that sleep disturbances may be related to insomnia or circadian issues.

**Methods:** Caregivers of 31 patients (19 children) with CdLS completed a sleep history questionnaire focused on sleep patterns and evening sleep behavior to screen for signs and symptoms of insomnia and circadian rhythm disorders.

**Results:** The mean age of participants was 14.5 years (range 0.6–37). Major difficulty in falling asleep (75% pediatric, 33% adult) and staying asleep (52% pediatric, 33% adult) was noted. Overall, time to sleep onset was 27.0 ± 17.6 min, however in those with stated sleep onset difficulty, average time to sleep was 37.8 ± 16.4 min (p = 0.002). The mean number of pediatric nighttime awakenings was 1.5 overall and 2.1 in those with stated sleep maintenance difficulties versus 0.7 and 1.5, respectively in adults. Children with CdLS tended to fall back asleep slower (61.8 min) than adults (14.9 min), but none of the comparisons between adult and pediatric sleep measures were significant. Greater than half of participants reported a family member with a possible circadian rhythm disorder.

**Conclusions:** Symptoms suggestive of insomnia or circadian rhythm disorder are prevalent in this cohort of children and
adults with CdLS. Adults may have less severe symptoms than children, suggesting some improvement over time although this study is underpowered for this analysis. Further studies are necessary to better characterize sleep disturbance in the CdLS population.

**Regulation of Transcription by Cohesin**

Dale Dorsett, Ziva Misulovin, Cheri A Schaaf, Avery Fay, Audrey Watson, and Maria Gauze

Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, Saint Louis, Missouri

In Drosophila, mouse and human cells, Nipped-B (*NIPBL*) and cohesin selectively bind and regulate many genes that control growth and development. Using genomic approaches, we find that at Drosophila cohesin-binding genes, RNA polymerase transcribes several nucleotides and then pauses before being induced to enter productive elongation. Cohesin does not physically block transcriptional elongation, and is not needed for polymerase pausing, but at many genes, negatively or positively affects the efficiency with which paused polymerase transitions to elongation. We are currently exploring the mechanisms by which cohesin controls transition to elongation. Supported by NIH grant GM055683.