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Cornelia de Lange syndrome and molecular implications of the cohesin complex: Abstracts from the 7th biennial scientific and educational symposium 2016

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Abstract

Cornelia de Lange Syndrome (CdLS) is due to mutations in the genes for the structural and regulatory proteins that make up the cohesin complex, and is considered a cohesinopathy disorder or, more recently, a transcriptomopathy. New phenotypes have been recognized in this expanding field. There are multiple clinical issues facing individuals with all forms of CdLS, particularly in the neurodevelopmental system, but also gastrointestinal, cardiac, and musculoskeletal. Aspects of developmental and cell biology have found common endpoints in the biology of the cohesin complex, with improved understanding of the mechanisms, easier diagnostic tests, and the possibility of potential therapeutics, all major clinical implications for the individual with CdLS. The following abstracts are the presentations from the 7th Cornelia de Lange Syndrome Scientific and Educational Symposium, June 22–23, 2016, in Orlando, FL, in conjunction with the Cornelia de Lange Syndrome Foundation National Meeting. In addition to the scientific and clinical discussions, there were talks related to practical aspects of behavior including autism, transitions, communication, access to medical care, and databases. At the end of the symposium, a panel was held, which included several parents, affected individuals and genetic counselors, and discussed the greatest challenges in life and how this information can assist in guiding future research. The Research Committee of the CdLS Foundation organizes this meeting, reviews, and accepts abstracts, and subsequently disseminates the information to the families through members of the Clinical Advisory Board and publications. AMA CME credits were provided by Greater Baltimore Medical Center, Baltimore, MD.

Keywords

behavior; CdLS; cohesin complex; cohesinopathy; de Lange syndrome; intellectual disability; transcription

Transcriptomopathies—A Growing Group of Transcriptional Regulatory Disorders With Phenotypic Overlap With Cornelia de Lange syndrome

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Mutations in structural and regulatory cohesin proteins including NIPBL, SMC1A, SMC3, RAD21, and HDAC8 are causative of CdLS. Cohesin, a multiprotein complex, plays a canonical role in regulating sister chromatid segregation during mitosis. A non-canonical role for cohesin in regulating gene expression is the likely mechanism underlying developmental disorders. Growing evidence supports a “transcriptome disruption model” for cohesinopathies and related diagnoses, including the implication of key transcriptional regulators (Mediator, Polycomb, CTCF, and others) with cohesin. Most recently our group

identified mutations in the *AFF4* gene, a critical component of the Super Elongation Complex (SEC) in a CdLS-like disorder (CHOPS syndrome), demonstrating regulatory interactions between Cohesin, the SEC and transcriptional elongation. A large number of genes/proteins are involved in the complexity of transcriptional regulation (from initiation, general transcription, elongation, pausing, backtracking, processing, termination, and associated epigenetic modification). Increasingly many of these genes, and the protein complexes they contribute to (cohesin, mediator, CTCF, TAF, SEC, CBP, SWI/SNF, ASX) are being implicated in human developmental disorders when disrupted. The term "transcriptomopathies" was coined by Yuan et al. (JCI, 2015). Interestingly, genetic disorders caused by mutations in components of the transcriptional machinery as well as in the proteins involved in epigenetic modification of the genome share many overlapping features (e.g., the Cornelia de Lange, Rubinstein-Taybi, Coffin-Siris, Bohring-Opitz, CHOPS syndromes, and others). Both reverse and forward genetic approaches have led to both insights into transcriptional regulation as well as the identification of novel disease genes. An overview of these disorders and their molecular and clinical interrelatedness will be discussed.

Insights Into Cornelia de Lange syndrome From Exome Sequencing

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Over the past decade, we have gained a great degree of understanding of Cornelia de Lange Syndrome and its molecular pathology. This began with recognition of mutations in *NIPBL*, and subsequently, other cohesin complex members and regulatory proteins in children with classic features of CdLS. This then led to our ability recognize that children with features suggestive of CdLS, but not classic typical features, could also have changes in some of these same cohesin genes, including *SMC1A*, *SMC3*, *RAD21*, and *HDAC8*.

Over the past few years, we have begun to develop additional knowledge: (1) About clinical features that do not strongly overlap CdLS, but are caused by mutations in the same genes; and (2) About additional genes with mutations that cause some clinical similarity to CdLS.

Much of this emerging knowledge has been gained from exome sequencing of a broad range of children with congenital or developmental abnormalities. For example, we have recently recognized that mutations in *SMC3* have a much broader range of clinical phenotypes that originally appreciated. In addition, while missense mutations in *SMC1A* can cause a clinical picture that has a great degree of overlap with classical CdLS, many children with *SMC1A* missense mutations have much milder features. To extend this knowledge, we have recently recognized that loss-of-function mutations in *SMC1A* may result in a clinical picture that has few facial features of CdLS, but causes a severe epileptic encephalopathy.

In addition to recognition of new phenotypes caused by mutations in known cohesin genes, the field has been able to identify other genes in which mutations cause phenotypes

suggestive of CdLS. The recent identification of mutations in *AFF4*, to cause a clinical picture of CHOPS syndrome is a good example of this. We have been working to elucidate additional genes and groups of patients in which identified variants may cause features that overlap with the facial and developmental features of CdLS. A number of these subjects have been identified via clinical exome sequencing, and we are working to identify additional children with changes in the same genes.

Some of the novel understanding we have gained on the range of phenotypes caused by mutations in known cohesin factors will be reviewed. In addition, some clinical disorders that have some phenotypic similarity to CdLS, but are caused by mutations in un- or loosely related genes will be outlined. Finally, some of the ways that these genes may result in overlapping clinical pictures will be speculated.

Transcriptional Regulation by Cohesion and Its Loader

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Sister chromatid cohesion (SCC) is crucial to ensure accurate chromosome segregation during mitosis. The cohesin complex mediates SCC, and recent studies show cohesin and NIPBL/Mau2 complex, a loader complex required for the loading of cohesin onto chromatin, as important players in transcriptional regulation. To analyze the precise mechanisms of transcriptional regulation by cohesin and its loader, we applied in vitro Pre-initiation complex (PIC) and Early Elongation Complex (EEC) assembly systems. In this system, we used the biotin-labeled DNA template, which contained 5xGAL4 DNA binding motifs, adenovirus late promotor sequence, and a part of luciferase gene. After binding of GAL4-VP16 activator protein to the DNA, PIC, and EEC assembly were induced by addition of the nuclear extract from HeLa cells. Each component of protein complex formed on template DNA was monitored by Western blotting and Mass-spec analysis. We found that PIC factors, mediator, general transcriptional factors and RNA polII, were recruited to the template, in an activator dependent manner. Further, we observed cohesin and NIPBL/Mau2-binding to the template, and their recruitments also depend on the activator. Precise step by step analysis is now underway to clearly determine the role played by cohesin and its loader during transcription. Based on the results we have collected so far, we propose that cohesin-loader and cohesin together regulate a step that controls re-activation of paused RNA polII nearby promotor.

RNA Binding Proteins Aid Association of Cohesin With Genes and Enhancers

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The cohesin complex mediates SCC and plays multiple roles in gene transcription, including facilitating long-range enhancer-promoter interactions. The Nipped-B protein that loads cohesin topologically onto chromosomes co-localizes with cohesin, and they both occupy active transcriptional enhancers and a large subset of transcribed genes.

Nipped-B and cohesin preferentially associate with genes in which RNA polymerase II pauses just downstream of the transcription start site, but it is unknown what factors determine which genes bind cohesin. Active genes that bind Nipped-B and cohesin are enriched for TG repeats downstream of the transcription start site in the non-template strand, raising the possibility that RNA-binding proteins aid their binding to specific genes.

We find that the TBPH and Lark RRM domain proteins interact with Nipped-B, and that TBPH broadly facilitates association of Nipped-B and cohesin with both active genes and enhancers. Lark aids Nipped-B and cohesin association with specific enhancers. These findings support the idea that sequence-specific RNA-binding proteins are an important factor in determining which genes and regulatory sequences associate with SCC proteins.

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High Sensitivity Sequencing in CdLS—HaloplexHs

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Mutations in genes for three core cohesin subunits (SMC1A, SMC3, RAD21) and in two cohesin-regulatory proteins (NIPBL, HDAC8) have been identified in CdLS probands. Mutations in the *NIPBL* gene have been identified in ~60% of "classical" CdLS and mutations in the other four genes account for ~5% of patients with a milder CdLS phenotype. To date, we have identified mutations in over 300 probands by CSGE/Sanger sequencing/MLPA/SNP array. Mutations including missense, nonsense, small deletions, and insertions, splice site mutations, and genomic rearrangements have been identified in these genes. In 30–35% of our cohort, a causative mutation has not been identified. This could be due to a number of possibilities including: (1) technical limitations (mutations are in regions of these genes not detected by currently used screening methodologies); (2) mosaicism with levels in blood (most commonly used tissue source for DNA diagnostics) is too low to detect or absent; (3) causative mutations lie in other genes yet to be discovered. The complexity of screening these genes and the need to screen all genes in every proband in a high-throughput manner, yet accurately and with a high depth of sequencing coverage is critical both for diagnostic purposes as well as discovery (to identify a cohort that will facilitate novel gene discovery for CdLS). In the last few years, the development of high-throughput technologies, such as next-generation sequencing (NGS), has allowed for the screening of a large number of genes simultaneously. This technique offers high sensitivity and efficiency, making the molecular diagnosis of genetically heterogeneous diagnoses, like CdLS, easier. We designed a HaloplexHs CdLS NGS gene panel that targets the five known CdLS genes

and seven new candidate genes and genes that cause disorders that phenotypically overlap with CdLS. Agilent's HaloPlexHs technology is a high-sensitivity target-enrichment system for NGS PCR that incorporates more than one million unique molecular barcodes for accurate detection of alleles at low frequencies. This would potentially be an excellent tool to detect hidden low level mosaic mutations in our cohort. This panel can be reliably used for the molecular diagnosis in known genes to identify causative pathogenic variants in mutation negative patients with CdLS and can serve as a primary screening tool. We are applying this tool to define the contribution of mosaicism to CdLS in mutation negative CdLS probands by deeper sequencing on samples from a variety of different tissues (saliva and blood derived DNA). Application of this diagnostic tool will allow us to define a CdLS cohort who are truly "mutation negative" to perform trio-based whole exome sequencing for discovery of novel CdLS causative genes.

PKR Activation May Contribute to Cornelia de Lange Syndrome

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NIPBL, a cohesin loader, has been implicated in transcriptional control and genome organization. Mutations in *NIPBL*, cohesin, and its deacetylase *HDAC8* result in Cornelia de Lange syndrome. We report activation of the RNA-sensing kinase PKR in human lymphoblastoid cell lines carrying *NIPBL* or *HDAC8* mutations, but not *SMC1A* or *SMC3* mutations. PKR activation can be triggered by unmodified RNAs. Gene expression profiles in NIPBL deficient lymphoblastoid cells and mouse embryonic stem cells reveal lower expression of genes involved in RNA processing and modification. *NIPBL* mutant lymphoblastoid cells show reduced proliferation and protein synthesis with increased apoptosis, all of which are partially reversed by a PKR inhibitor. Non-coding RNAs from an *NIPBL* mutant line had less N^6-Methyladenosine modification and activated PKR activity in vitro. This study provides insight into the molecular pathology of Cornelia de Lange syndrome by establishing a relationship between *NIPBL* and *HDAC8* mutations and PKR activation. We continue to explore whether PKR activation may contribute to developmental and behavioral defects in the *Nipbl*$^{+/-}$ mouse model for Cornelia de Lange syndrome.

Study of Roberts Syndrome With Mouse and iPS Cell Models

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Roberts syndrome (RBS) is an autosomal recessive disorder characterized by growth retardation, craniofacial abnormalities, and tetraphocomelia. Cellular alterations in RBS include lack of cohesion at the heterochromatic regions around centromeres and the long arm of the Y chromosome, reduced growth capacity, and hypersensitivity to DNA damaging agents. RBS is caused by mutations in *ESCO2*, which encodes a protein belonging to Eco1/Ctf7 family of acetyltransferases. ESCO2 is involved in regulating SCC. It has been found that the molecular mechanism underlying RBS involves loss of ESCO2 acetyltransferase activity, but few disease models are available to study the pathogenesis. To explore the pathological mechanism of RBS, we have generated a conditional-knockout (CKO) mouse model and patient-specific induced pluripotent stem cells (iPSCs). Using the *Prx1*-Cre; *Esco2*$^{f/f}$ mice, we are able to delete *Esco2* in early limb bud mesenchyme and in a subset of craniofacial mesenchyme. Embryos at 11.5–13.5 showed shortening of the forelimbs and hindlimbs, which resembles bilateral symmetric limb reduction in Robert syndrome patients. Reduced or absent expression of Sox9 was observed in limb buds of CKO embryos at E11.5, indicating defective cartilage development. Absence of Esco2 evoked excess cell death in limb buds by TUNEL assay, while cell proliferation was not significantly changed in the affected mesenchyme. Abnormal nuclear morphology was detected in limb buds of *Prx1*-Cre; *Esco2*$^{f/f}$ CKO mice with Hoechst 33258 staining, similar to the morphology of the nuclei with blebs that develop into micronuclei in RBS patients. In addition to the mouse model, iPS cells have been generated by reprogramming fibroblasts from two patients with RBS and healthy controls. These iPS cells can be used for studying the cellular defects of *ESCO2* mutation during differentiation and drug discovery. The mouse and iPS cell models can help us reveal the important role of *ESCO2* in development and decipher the mechanism of RBS.

Characterization of Limb Differences in Children With Cornelia de Lange Syndrome

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Cornelia de Lange syndrome (CdLS) is a well-described, multi-system, developmental disorder characterized by dysmorphic facial features, growth and cognitive deficits, and cardiac, gastrointestinal, and limb anomalies. The limb defects seen in CdLS can be mild, with small feet or hands only, moderate, or can be severe, with variable reduction defects involving primarily the ulnar structures and ranging from mild hypoplasia of the 5th digit to complete absence of the forearm. The upper limbs are typically much more involved than the lower extremities with foot involvement generally manifesting as small feet, 2–3 syndactyly of the toes, and shortened 4th metatarsal. The upper limb involvement is often asymmetric. The findings in our cohort of 378 patients show that a consistent pattern of

defects in laterality and symmetry are seen in CdLS with an increased severity of right-sided limb defects in probands with asymmetric limb defects. Additionally, our analysis suggests that limb defects correlate with additional systemic structural anomalies and more severe cognitive outcomes. Characterization of the limb differences in children with CdLS may provide a tool to assist in genetic counseling and determining prognosis. This presentation will review the limb involvement in a large cohort of individuals with CdLS assessing the correlation with molecular etiologies, symmetry, additional structural birth defects, and cognitive outcomes.

Cohesin's Role in the Maintenance of Pluripotency, Hematopoietic Progenitor Cells, and Differentiated Megakarycoytes

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The cohesin complex, and accessory regulatory proteins, plays a critical role in several basic cellular processes including its canonical role in SCC as well as several more recently described roles including DNA repair, long-range chromosomal architectural integrity, stem cell maintenance, and pluripotency and regulation of gene expression. The interest in cohesin's role in transcriptional regulation was heightened after mutations were identified in the human *NIPBL* gene in Cornelia de Lange syndrome (CdLS), a multisystem developmental disorder. NIPBL (Scc2 in yeast) is a cohesin regulatory protein that plays a critical role in the loading and unloading of the cohesin complex onto chromosomes. Mutations in additional cohesion structural (SMC1, SMC2, Rad21) and regulatory (HDAC8) subunits were also found to cause CdLS. Subsequently mutations in other cohesin related proteins have been identified in other developmental disorders collectively termed "cohesinopathies." Clinical manifestations of CdLS include intellectual disability, growth retardation, craniofacial abnormalities including cleft palate, limb defects, gastrointestinal defects, cardiac, and hematopoietic abnormalities. Our work seeks to advance our understanding of cohesin's role in the regulation of gene expression and sister chromatid segregation in the undifferentiated state, and during normal and pathological hematopoiesis, in particular, megakaryopoiesis. We have generated three iPSC lines from CdLS patients carrying endogenous mutations in cohesin-associated gene, *NIPBL*. We are comprehensively investigating (1) The mechanism by which cohesin controls global gene expression and sister chromatid biology in undifferentiated iPSCs and during hematopoietic developmental specific stages and attempting to (2) recapitulate the CdLS-related hematopoietic disease phenotype in patient derived iPSCs to model thrombocytopenia. Analysis of gene expression

in the undifferentiated state shows more than 1,500 differentially expressed genes in *NIPBL* cells compare to controls, with post-translational modifications, cell cycle, gene expression, and DNA repair, recombination and replication among the most significant processes altered. We found that hematopoietic progenitor cells (HPCs; CD41+/CD235+/CD43+) generated from *NIPBL* iPSC had a higher proportion (84% vs. 75%) compared to controls, respectively. These HPCs were then induced to generate lineage committed megakaryocytes cells (MEGs, CD41+/CD42+), and we observed no difference in MEG proportions from *NIPBL* and control HPCs (76% vs. 79%, respectively). iPSC derived HPCs and MEGs had reduced *NIPBL* mRNA expression (~18% decrease) relative to control cell lines. We have focused on the CdLS mutant gene *NIPBL*, which has served as the framework to develop an infrastructure where our methodologies can be applied to other cohesin genes and the related cohesinopathies to study changes in the transcriptome and epigenome.

Challenges Associated With Feeding in Individuals With Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS), also known as Brachman-de Lange syndrome, is a congenital syndrome in which affected individuals exhibit atypical facial, physical, and developmental characteristics associated with challenges in oral feeding. Physical differences can impact feeding. Children with micrognathia, small teeth, and a weak bite are at risk for difficulty with oral intake and management of food, and prolonged length of feeding. Cleft palate is associated with nasal regurgitation of food, and food can collect in a highly arched palate. Feeding aversions are linked to gastroesophageal reflux, tube feeding, autistic tendencies, and sensory issues. Delayed physical growth, gastroesophageal reflux disease, and possible failure to thrive may be associated with poor nutritional intake. Speech-language delays, apraxia of speech, sensory processing disorders, autistic tendencies, developmental delays, and self-injurious behaviors are other common sequelae.

Because feeding issues are prevalent among individuals with CdLS, specific feeding difficulties of 83 affected individuals, age 3 months through 36 years, were investigated via observations of feeding behaviors and information reported by caregivers. Significant findings included an increased incidence of 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. Additional feeding concerns included difficulty sucking, swallowing, chewing, and biting; consumption of only small bites; lack of transition to solid foods; lack of transition to cup drinking; selective eating or messy feeding habits; and slow feeding. Primary feeding concerns reported by caregivers included transitioning from tube to oral feeding, promoting eating of solid foods and foods of various textures, encouraging better overall eating habits, and developing appropriate feeding techniques. Health concerns that may be associated with feeding difficulties included allergies and lactose intolerance, and an increased incidence of colds, ear infections, pneumonia, sinusitis, fevers, and sepsis.

Treatment techniques to improve oral feeding depend on the needs of the individual and the reasons for the oral feeding difficulties. Best practices generally include providing oral stimulation 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 introduced, 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, etc. When the individual can safely feed orally, semi-solids typically are introduced first, then solids and liquids. If an individual cannot safely swallow without choking, coughing, gagging, retching, or aspirating, the food's thickness can be adjusted. Individuals who cannot safely manage liquids sometimes can tolerate soft solids or pureed foods. Initially introduce only one texture until feeding comfortably and safely. Next, modify the texture by varying its consistency, then change the flavor or temperature. Many individuals express an initial preference for bland foods and later move toward stronger flavors; some prefer the opposite. Before attempting feeding therapy, rule out food allergies. All feeding should occur in a comfortable, friendly environment.

Use of Blenderized Diet for Tube Feeding in Patients With Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome (CdLS) is a multisystem developmental diagnosis with variable growth, cognitive, craniofacial, limb, gastrointestinal, cardiac, and other systemic abnormalities. Individuals with CdLS present with significant gastrointestinal complications and tend to exhibit high rates of gastroesophageal reflux, poor oral intake, and malnutrition. Many children undergo Nissen procedures and feeding tube placement to ameliorate symptoms and to help achieve appropriate nutrition requirements necessary for growth and development. Despite these interventions, symptoms can still persist and have a negative effect on the child's health impacting not only the child's but also the family's quality of life. A significant percentage of individuals with CdLS receive the majority if not all of their food via tube feeding. Many individuals with CdLS who have long-term reliance on tube feeding and liquid diets have consistent visceral pain, discomfort, and behavioral issues. Traditionally, commercial formulas were used as a standard means of providing feeds via g-tube to ensure adequate nutrition and caloric intake. More recently, parents of children with CdLS who have g-tubes are reportedly using alternative diets such as pureed or blenderized diets where food and liquids are blended and given directly through the tube. Anecdotal evidence from families cared for at the Center for Cornelia de Lange syndrome and Related Diagnoses at The Children's Hospital of Philadelphia suggests that individuals with CdLS when transitioned from formula to a blended diet exhibit less frequent symptoms of retching

and gagging and are generally in less discomfort. There is limited data in the scientific literature analyzing the use of blenderized diets for tube feeding.

Currently, many dieticians have limited experience with blended diets and are hesitant to recommend pureed food over formula given a relative lack of standardized nutritional values for blended diets. Use of a blended diet may be beneficial to individuals with CdLS, especially those who continue to exhibit overt gastrointestinal symptoms, such as reflux and retching, which affects overall quality of life and long-term could lead to damaging side effects such as chronic esophagitis and Barrett's esophagus. Parents of individuals with CdLS using tube feeding are being surveyed to formally evaluate the benefits and disadvantages of implementing commercial versus blenderized diet. Through this research we aim to provide guidance to families and clinicians in understanding the benefits and risks of using blenderized diets and to optimize and improve the quality of care provided to patients with CdLS.

In Vitro Modeling of Cardiac Development Using CdLS Patient-Specific Induced Pluripotent Stem Cells

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Congenital heart disease (CHD) is the most common life-threatening birth defect in humans affecting approximately 1% of newborns. The study of CHDs through animal models has provided great insight into our understanding of cardiac development; however, our knowledge of how certain genetic mutations cause CHD in humans is limited given the scarcity of available embryonic tissues. Cornelia de Lange syndrome (CdLS) is one of a group of disorders known as cohesinopathies, caused by disruption of the normal function and regulation of the cohesin complex—a key regulator of gene expression during development. Nearly 50% of CdLS probands have structural congenital heart defects (pulmonic stenosis, atrial septal, and ventricular septal defects being the most common) or minor cardiac findings. CdLS is a dominant multisystem disorder caused by heterozygous mutations in cohesin structural and regulatory proteins, most commonly in NIPBL (>60% of cases), which is required for the loading and unloading of cohesin onto DNA. While cohesin's canonical role in sister chromatid segregation is not disrupted in CdLS, our studies support a dysregulation of gene expression as the major contributor to the phenotype. It has been shown that as little as a 15% decrease in NIPBL expression can lead to multiorgan defects, including disruption of gut and heart development during embryogenesis. *NIPBL* regulation of gene expression is critical in the molecular and cellular mechanism responsible for proper functional cardiomyocyte generation and disruption of these processes likely

result in the CHDs seen in CdLS. Through the reprogramming of somatic tissues, most commonly fibroblasts and peripheral blood mononuclear cells, we generated induced pluripotent stem cells (iPSCs) from three patient samples harboring *NIPBL* mutations. Mutational analysis has confirmed that generated iPSC lines maintain the original *NIPBL* mutations. To establish a model for studying early cardiac development, we differentiated NIPBL mutant and normal control cell lines into in vitro derived cardiomyocytes essentially creating a model for examining cohesin's role in cardiogenesis. All cardiomyocyte generated using this protocol expressed cardiac specific proteins quantified by flow cytometry (CTNT2, VCAM1, SIRPA) and immunofluorescence (CTNT2, NKX2.5, α-Actinin). Cardiomyocyte generation was robust in both control and CdLS patient lines with some variation in cardiomyocyte cell number and proportion of cells (70–93% vs. 70–75%, respectively). Gene expression analysis confirmed decreased NIPBL levels in CdLS patient lines when compared to that of controls. RNA sequencing was performed on all cardiomyocyte samples isolated after 15 days of in vitro development. Our data show a model amenable for cardiomyocyte differentiation of *NIPBL* mutant iPSCs that can be used for disease modeling of cardiovascular development while establishing a human model platform for testing therapeutic agents.

Neural Crest Origin for Cohesinopathy Heart Defects

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Mutations in *NIPBL*, or in subunits or regulators of cohesin, cause a spectrum of disorders in humans known as the "cohesinopathies." Cohesinopathies, including the best known example Cornelia de Lange Syndrome (CdLS), are characterized by broad spectrum, multifactorial developmental anomalies. Heart defects occur at high frequency and can reach up to 30% in CdLS. The mechanisms by which heart defects occur are enigmatic, but assumed to be developmental in origin. We depleted cohesin subunit Rad21 by 70–80% in a zebrafish cohesinopathy model. The hearts of Rad21-depleted animals were smaller, often failed to loop, and functioned less efficiently than size-matched controls. Functional deficiency was accompanied by valve defects and reduced ejection fraction. Interestingly, neural crest cells failed to populate the heart and instead exhibited a wandering behavior. These cells subsequently failed to condense correctly into pharyngeal arches. Transcriptome analysis revealed that Wnt pathway, chemokine, and cadherin genes are dysregulated at the time of cardiac neural crest development. In pre-gastrulation zebrafish embryos, Rad21 depletion led to disrupted formation of nucleoli and RNA Polymerase II transcription

factories, suggesting that cohesin-deficient cells have additional problems with global nuclear organization, transcription, and translation. Our results give insight into the etiology of heart defects in the cohesinopathies, and raise the possibility that mild mutations in cohesin genes may be causative of a fraction of CHD in human populations.

Transcriptional Analysis of CdLS Patient-Specific Neural Cells Highlight Early Cell Cycle Exit in Developing Cortical Cells Derived From iPSCs

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Cornelia de Lange Syndrome (CdLS) is a rare dominant genetic disease that leads to structural, developmental, cognitive, and neurological abnormalities. Mutations associated with the cohesin complex or associated proteins result in variable clinical presentations including intellectual disability (ID, syndromic, and non-syndromic), altered sleep and behavioral challenges, anxiety, attention deficit hyperactivity disorder, and autistic-like characteristics. While most patients harbor mutations in the gene for NIPBL (>65% of cases), a protein required for the loading and unloading of cohesin onto DNA, mutations in genes in or associated with the cohesin complex (*SMC1*, *SMC3*, *RAD21*, *HDAC8*) account for a significant number of cases (~15% of cases). The size and surface area of the brain are critical elements associated with intellectual abilities. Small heads are a common feature associated with patients with CdLS. The neocortex is considered a key part of the mammalian telencephalon and a region that enables higher cognitive function and brain size. The development of the neocortex involves generation and amplification of neural progenitor cells and transit-amplifying cells that form the proliferative region of the ventricular epithelium. These progenitor cells produce the layers of the subcortical structures composed of projection neurons necessary for neocortical formation and functions associated with cognitive ability. A main drawback to studying neurobiology clinically is the inaccessibility of tissue samples for genetic, epigenetic, and gene expression experiments; therefore, developing a roadmap to the complex neural development through basic human tissue culture models is needed. We have developed an in vitro model that uses CdLS patient-specific induced pluripotent stem cells (iPSC) carrying *NIPBL* mutations to study the role of cohesin on early stages of neuronal patterning, specifically focused on anterior forebrain development. Our data show that NIPBL levels are reduced in all stages (neuro-epithelium, neural progenitors, and post-mitotic pyramidal neurons) of neural patterning. Transcriptional and protein analysis at the neuronal progenitor stage show that CdLS samples harboring *NIPBL* mutations have abnormal expression of key neuronal markers compared to control samples. In particular, *PAX6*, *OTX1/2*, *EMX2*, have increased expression after 12 days of forebrain patterning, with no change in forebrain marker

FOXG1, and a lower expression of cell cycle regulator, *CCDN1*. The observed impaired expression of important developmental genes at early stages suggests a model of premature neuronal differentiation, cell cycle exit, and early post-mitotic neuron generation, which may help explain the observed microcephaly and cognitive disability in CdLS patients.

Coordination of Rare Diseases at Sanford (CoRDS)

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Background: The Children's Health Research Center at Sanford Research has established an international rare disease patient registry named Coordination for Rare Diseases at Sanford (CoRDS) to advance rare disease research. CoRDS houses data on individuals with a rare disease diagnosis and those awaiting diagnosis. CoRDS also partners with patient advocacy groups (PAGs) to provide access to a platform to conduct natural history and other research studies. CoRDS partnered with the Cornelia de Lange syndrome (CdLS) Foundation in May of 2014. The mission of this partnership is to collect genetic, diagnostic, medical, and demographic data.

Methods: The key operational components for the CoRDS registry include data collection, data management and data dissemination. Coordination with the CdLS Foundation has provided CoRDS the ability to collect data on diagnosis/assessments, birth history, family/reproductive history, and various organ systems. PAGs that partner with CoRDS have an opportunity to sign an agreement to access data for non-research purposes when participants provide consent. By signing this agreement they may also be involved in the review of the researchers trying to access their data.

The CdLS questionnaire collects disease-specific data as well as the Office of Rare Disease Research Common Data Elements to help harmonize the data collection process. Participants are provided with data sharing preferences which allow them the opportunity to share data with existing registries and the CdLS Foundation.

Results: As of February 1, 2016 CoRDS 74 fully enrolled participants diagnosed with CdLS. Of the 74 enrolled participants 43.2% are ages 0–10, 25.7% are 11–20 years old, 18.9% are 21–30 years old, 10.8% are 31–40 years old, and 1.4% are 41–50 years old. There are 31 states and two countries excluding the United States with enrolled individuals. Determination of diagnosis is done by physical examination (58.7%), genetic analysis (27.2%), and CT/MRI imaging (6.6%).

Discussion: Collecting and collating rare disease data and natural history data for specific diseases offers the opportunity to perform a comparative analysis to better understand and treat these diseases. In order for cures, treatments, and therapies to be developed mechanisms of the disease must be understood. The CdLS Foundation registry and CoRDS are gathering, collating, and disseminating this data to accelerate research for those affected by Cornelia de Lange syndrome.

WaihonaPedia Platform: A Source for Knowledge Development and Research for Cornelia de Lange Syndrome

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All people are unique individuals. A very small number of the babies born are even more unique because they are born with a rare difference (syndrome). Because of this, general recommendations for specific syndromes do not always fit for the particular condition, especially a rare one like Cornelia de Lange syndrome (CdLS). There is much energy put in dealing with common aspects of wellbeing, mostly because our society wants to do things efficiently, and conditions that are common have priority. One of the reasons that these common approaches seem to fail for children with CdLS is the fact that they are specifically different! We wanted to develop a platform where families, caretakers, teachers, doctors, therapists, and researchers could see, discover, and learn paths to more happiness and more well-being for an individual with a rare syndrome, such that it would be possible to (1) Find well-developed treatments and advice to build a personal education/careplan; (2) Ask questions to enrich this care plan; (3) Collect data that can be shared with educators, doctors, and researchers; and (4) Reach out to other families and experts to discuss and learn from each other and improve the knowledge and practices available (and where needed to create the missing pieces). This is based on the structure and trust build into foundations, which are communities of families for families.

This platform will be called WaihonaPedia, from Waihone: "treasure/dear" from the Hawaiian language. The platform will be owned by families or caretakers, and professionals will be invited to participate. It will become a source for knowledge development and research, as well as improving discussions between families and professionals. The WaihonaPedia platform will support the following four functions: (1) Knowledge and practice development, including ease in readability of the protocols; (2) Closer look at shared stories from families' experiences including being responsible for editing, lack of harassment, no violation of copyright or other laws with published sources, and "doing no harm" regarding the infrastructure; (3) Questions put forth and answers by experts, which might generate areas for research; and (4) Questionnaires and/or score cards to compare the child's findings with others affected, under the auspices of recognized experts. Families will be able to capture data about their child and share it with peers, school, hospital, or researchers.

This project was proposed by eight parent foundations for rare diseases, approved/financed by the Dutch Ministry of Health, and fits into the European Union plan for Rare Diseases. It will run from 2016 to 2019. One of the eight is the CdLS Vereniging, with collaboration with the AMC Cornelia de Lange Centre, an approved expertise center for Cornelia de Lange syndrome, Pitt-Hopkins syndrome, Marshall-Smith Syndrome, and Rubinstein-Taybi

syndrome. The project is based on the prototype platform www.cdlsworld.org, where the World Federation of Cornelia de Lange syndrome patient groups have been collaborating for the past 10 years. Many of its functions are based on initiatives started by the USA CdLS Foundation, including the Ask the Expert section. We will present our initial proposal and progress to date and discuss our plans for the future.

Autism Spectrum Disorder in Cornelia de Lange syndrome: Prevalence, Profile, and Developmental Trajectory

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A heightened risk of Autism Spectrum Disorder (ASD) and related characteristics has been reported in a number of genetic syndromes. The presentation of these characteristics is often described as being atypical to idiopathic ASD and these atypicalities might reflect differences in underlying social-cognitive mechanisms and aetiological pathways.

In Cornelia de Lange syndrome (CdLS), reported prevalence rates of ASD range between 50 and 70%. Comparisons between individuals with CdLS and those with idiopathic ASD confirm that while there are broad behavioral similarities, subtle differences within specific areas of communication, social interaction, and repetitive behavior are evident. For example, social anxiety and selective mutism are unusually prominent in CdLS, while some aspects of non-verbal communication (e.g., gesture use) appear to be relatively less impaired. Moreover, longitudinal studies indicate that there may be significant changes in the severity of these characteristics over time, that are not evident in the broader ASD population. These findings have implications for the assessment, diagnosis, and intervention of ASD and related characteristics in individuals with CdLS and highlight some important methodological considerations for future research.

In this talk, the prevalence of ASD in CdLS will be reviewed. The profile of ASD that is characteristic of individuals with CdLS and the broader social phenotype associated with the syndrome, will be described, including emerging evidence from eye tracking studies. Finally, based on the findings from a seven year follow up study of individuals with CdLS, the importance of continued ASD assessment throughout the lifespan will be highlighted.

Application of Eye-Tracking to Understand Social and Emotional Differences in Individuals With Cornelia de Lange Syndrome

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Eye-tracking is a robust and powerful technology, which allows researchers to better understand how individuals visually examine, explore, and think about various types of stimuli. Eye-tracking may be particularly valuable for furthering our understanding of individuals who are nonverbal or minimally verbal. However, this technology has only begun to be applied with individuals with rare syndromes. This presentation describes the first reports on the application of eye-tracking technology for the study of individuals with Cornelia de Lange syndrome (CdLS). Specifically, we examine scanning of face features (e.g., eyes, mouth), facial emotions (e.g., happy, disgust), and participant preferences for watching videos of human versus non-human actions and activities. The aim of this research is to determine whether or not the looking patterns of individuals with CdLS differ from other individuals in ways that might suggest or reflect differences in social anxiety, emotion perception, or social interest. Comparison groups in these studies include typically developing individuals, as well as individuals with Autism, fragile X Syndrome, and Rubinstein–Taybi Syndrome. Contrary to our hypothesis, individuals with CdLS did not exhibit reduced attention to the eyes of faces when compared with these groups. They also did not differ from any of the other groups with regards to their ability to distinguish faces expressing different emotions from one another. However, individuals with CdLS did take longer to fixate to videos of people moving toward them versus videos of objects moving towards them than did the other participant groups. This latter finding suggests that attentional prioritization of socially salient stimuli is reduced in those with CdLS, which may be related previous descriptions of social anxiety and/or a reduced ability to interact with others in this population. We are currently planning further eye-tracking studies of individuals with CdLS in order to identify methods and procedures which might ultimately aid in assessing and supporting these individuals and their families.

Sensory Deficits and Autistic Features in Children With Cornelia de Lange Syndrome: The Role of Tactile Sensitivity

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Introduction: Cornelia de Lange Syndrome (CdLS) is a developmental disorder characterized by distinct facial features, neuro-developmental deficits, and short stature (Cheung and Upton, 2015). Children with CdLS also have autism spectrum disorder (ASD) traits, characterized by sparse verbal output, and obsessive-compulsive/repetitive behaviors (Srivastava, 2014). Sensory sensitivity deficits are common in children with CdLS, including tactile, auditory, gustatory, and vestibular deficits. In order to better understand the relationship between sensory deficits and autistic features in children with CdLS, a well-characterized sample of children with CdLS phenotyped on both domains is examined in order to elucidate these relationships.

Methods: A sample of 41 children, ages 7–17 participated in clinical interviews by phone with a child psychiatrist and completed self-report questionnaires. Autistic traits were obtained through the Children Autism Rating Scale (CARS) (Schopler, 1988). From CARS items, 5 facets were derived: social cognition, verbal, repetitive/ritualistic, affective/behavioral and sensory processing. Sensory sensitivity was assessed with the self-report Sensory Profile, a caregiver questionnaire consisting of 125 questions, with the following subscales: Tactile, Auditory, Visual, Vestibular, Multisensory and Oral (Dunn, 1997).

Results: The presence of sensory processing deficits increases the severity of the ASD features. Tactile sensory processing is the most significant predictor of autistic features, affecting several ASD domains: social cognition ($p = 0.002$), verbal output ($p = 0.006$), and obsessive-compulsive/repetitive behaviors ($p = 0.006$). Other sensory deficits are not related to ASD features. However, the multisensory Dunn subscale, which reflects various developmental deficits, was associated with low verbal output ($p < 0.05$) and deficits in social cognition ($p < 0.05$) in children with CdLS.

Conclusions: Tactile sensitivity is a unique risk-enhancing trait for ASD features in children with CdLS. Recent research showing that amygdala-mediated lack of habituation underlies sensory deficits may explain the association of these phenotypic features with ASD traits in CdLS.

Self-Injurious Behavior Profiles in Cornelia de Lange and Fragile X Syndromes

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Background: Self-injurious behaviors are among the most concerning behaviors in individuals with developmental disabilities. Individuals with Cornelia de Lange Syndrome (CdLS) have elevated levels of repetitive, self-injurious behaviors in comparison to children with typical development and/or other intellectual disability syndromes. In this study we examined the relationships between independent communication skills, autism diagnosis, anxiety, and age in CdLS and fragile X Syndrome (FXS).

Methods: 37 individuals with CdLS (51.1% male, mean age 11.6 yrs. [SD = 3.9]) and 30 individuals with FXS (82.2% male, mean age 12.6 yrs. (SD = 4.8)) were assessed by parental report on two behavioral measures. The Spence Children's Anxiety Scale (SCAS) and a questionnaire about the frequency and topography of self-injurious behaviors were conducted. Self-injurious behaviors were coded to the Repetitive Behavior Scale-Revised, Self-Injurious Behavior Subscale.

Results: Individuals in both CdLS and FXS groups who had been diagnosed with autism had significantly higher rates of self-injurious behaviors than those who did not have autism diagnoses at the $P < 0.05$ level. In both groups, individuals who could not communicate independently also had significantly higher levels of self-injurious behaviors than those who could communicate effectively at the $P < 0.05$ level. In the CdLS group there was a negative correlation between self-injurious behaviors and anxiety ($r = -0.23$), whereas the FXS group showed a positive correlation between self-injurious behavior and anxiety ($r = 0.19$). Age and self-injurious behaviors did not correlate significantly in either group.

Conclusions: Results indicate a stronger need to support individuals with CdLS who have autism diagnoses and challenges with communication deficits.

Use of Picture Exchange Communication to Improve Communication for Nonverbal and Minimally Verbal Individuals With Cornelia de Lange syndrome

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Background: Disruptive behaviors remain a challenging area for individuals with Cornelia de Lange syndrome (CdLS), given the presence of mild to profound intellectual disability, low adaptive skills, severe expressive language impairment, and autistic features. Despite the impact of these behaviors, there remains very little research-based information to guide specific developmental interventions for individuals with CdLS, particularly in the area of communication. One such intervention previously studied in autism spectrum disorder is a picture communication system (PCS), which involves teaching nonverbal and minimally verbal individuals to communicate by seeking out communicative partners and exchanging pictures with them. In this study, we aim to evaluate the effectiveness of a specific PCS

called Picture Exchange Communication System (PECS) for improving communication in nonverbal individuals with CdLS.

Methods: We are conducting a prospective intervention case study series involving nonverbal/minimally verbal individuals with CdLS (ages 3 years through adulthood). All participants are undergoing baseline behavior and developmental evaluation including: Vineland Adaptive Behavior Scales (VABS; parent interview version), Social Communication Questionnaire (SCQ; parent), Aberrant Behavior Checklist (ABC; parent), Child Autism Rating Scale (CARS; provider interview version). In addition, baseline video recordings of communication skills are being taken. The intervention consists of standardized PECS parent training provided to the family across several home visits. During a follow-up visit after 5–9 months, participants will undergo a repeat post-intervention communication assessment video recording and repeat VABS, SCQ, ABC, and CARS evaluations. Comparison between pre- and post-evaluations of (1) video demonstrating specific communication tasks and (2) scores from the behavioral instruments will help determine effectiveness of the intervention.

Results: Five participants have been enrolled in the study and completed baseline developmental assessments and video recordings of their communication skills. PECS training is ongoing. Specific progress will be discussed.

Conclusions: Given that there are no published reports of individuals with CdLS learning communications interventions such as PECS, this research is an exciting first step toward identifying strategies for improving communication, which can be quite challenging, and subsequently reducing disruptive behaviors in individuals with CdLS. Initial results are promising.

Transitions: It is Never Too Early to Start Planning

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Transitions connote change from one set of situations to another over a period of time. For many, looking forward to what comes next is exciting, for others, the thought is daunting and a time of stress. We agree that transitions do not occur over night and they allow for a process to prepare for the next stage in life.

For all children, they transition from home to school, and then from the various grades and levels in school toward graduation and preparation for adult life with perhaps advanced training and education, work and the goal of independence. We have seen this from the era of No Child Left Behind to the Race to the Top and now the College and Career Ready mantras.

For families with children with special needs, they transition from home-based services to school settings, from family-focused IFSPs to child-focused IEPs, to Transition Plans and

from the umbrella of rights under IDEA to a more limiting focus of Adult Services. Families learn to navigate the differences across an array of service agencies that include the Department of Health, Department of Education, and Vocational Rehabilitation—hopefully. The goal again is often work and independent or supported life in the community.

For families with children, youth and young adults with complex needs, we need to define a vision of the future that may or may not include competitive employment and where a person's success is not measured by a paycheck, but by a rich quality of life. For this to occur, it is never too early to start planning!

Person-Centered Planning is a process that allows the family and a group of people who know the child, youth or young adult to come together to focus on their gifts and talents, interests, and skills, what works and does not work, needs and most importantly dreams to help develop plans to make dreams become reality.

When parents were asked what were important transition goals for their children, their answers are the basis of our presentation: (1) Access the world for a better quality of life; (2) Meaningful activities throughout the day; (3) Active participation in their communities; (4) Enjoyable relationships and friendships; (5) Continuous opportunities to learn new things; and (6) Opportunities for control through choice.

In this session, we will explore some of the feelings families have around transitions, discuss developing self-determination, and life skills, provide a frame for the larger support network for persons with Dual Sensory Loss or Deaf-Blindness and highlight free supports and resources. We will provide information on a person-centered planning process to help families plan for the future.

Behavior, Development, and Clinical Findings in Cornelia de Lange syndrome: Correlations With Findings on MRI of the Brain

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Neurodevelopmental and behavioral issues are prominent features of Cornelia de Lange syndrome (CdLS). We previously compared central nervous system findings on brain MRI retrospectively to assessments of behavior by the Aberrant Behavior Checklist (ABC) (Roshan Lal et al., 2016). In evaluating brain MRIs from 15 individuals with CdLS, 10 patients (67%) had structural abnormalities and of these, 60% had “clinically significant” behaviors on the ABC. Although not statistically significant, the more severe the findings were on the MRI, the more “clinically significant” were the ABC results. “Elevated” scores on the irritability and lethargy subscales appeared to be associated with cerebral atrophy and/or more severe changes of the brain on the MRIs, suggesting that these neuroanatomic findings could correlate with some features of social isolation, depression and/or autistic features in CdLS. In addition, abnormal behaviors on the ABC were noted in 80% of the five individuals with normal brain MRIs.

Correlating abnormalities of the brain with phenotypic findings could help provide prognosis, make medical recommendations, or guide preventative measures, and no previous longitudinal study has attempted this. We have continued this study, assessing additional MRI’s of the brain and expanding the phenotypic information to include other specific behavioral diagnoses, as well as intellectual abilities and molecular information if available. We calculated the CdLS clinical severity score (Kline et al., 2007), and compared it to the degree of involvement of the brain MRI (Roshan Lal, et al., 2016) for each patient. In general, the higher the clinical score, indicating increased involvement of multiple body systems, the more severe the findings on MRI of the brain. This was true for the presence of autism as well. The presence of seizures had no correlation with brain findings. Some specific behavioral entities appear to correlate with certain findings on MRI. Further correlations and trends will be discussed.

COMPASS-31 Questionnaire Screening in Individuals With Cornelia de Lange Syndrome

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Cornelia de Lange Syndrome (CdLS) is characterized by stereotypical facial features, growth failure, limb abnormalities, and gastroesophageal dysfunction. CdLS may be caused by mutations in several genes that disrupt gene regulation during critical stages of early development. Affected individuals often experience acute episodes of pain or discomfort with no detectable physical correlation, whereas others seem to exhibit a lack of pain sensitivity. The extent of autonomic dysfunction in CdLS patients has not been well

characterized. Previously, questionnaire data were collected from 26 individuals with CdLS, and intradermal histamine testing was performed on 24. These results suggested that individuals have a high tolerance or delayed reaction to pain, skin blotching, and other symptoms of autonomic dysfunction (Kline et al, 2002). At least one individual has been found to have a mild peripheral neuropathy, and anecdotally, some individuals achieve some benefit from medications that address peripheral neuropathy.

We assessed symptoms of autonomic dysfunction in individuals with CdLS using the COMPASS-31, a validated survey tool for autonomic symptoms as a base for further projects to study the role of autonomic dysfunction. Individuals with CdLS (or family member if individual unable to complete) aged 8 or older were asked to complete the COMPASS-31 survey tool. The survey link was posted on the CdLS Foundation website and was emailed to individuals/families that had given permission to be contacted. Data were collected in the RedCap web-based application. A score of 11 and above was used to indicate at least mild autonomic dysfunction with a score greater than 26 suggestive of moderate to severe dysfunction. We collected 65 surveys, of which 34 were on females and 31 on males with ages ranging 8–51, and with mean age of 22.4 years. The results showed that 81% of individuals surveyed had at least mild autonomic dysfunction and 26% had moderate to severe dysfunction.

Based on survey data, individuals with CdLS may experience autonomic dysfunction affecting quality of life. Further investigation is necessary with direct autonomic testing to confirm these results. We are in the process of evaluating individuals with CdLS 8–18 years for autonomic function, including the Autonomic Reflex Screen with a tilt table test, QSweat measurements, and thermoregulatory sweat tests. A deidentified database will be established with the results. This study is ongoing.

Heterozygous Drosophila Models of Cornelia de Lange Syndrome

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Individuals with Cornelia de Lange Syndrome (CdLS) display diverse developmental deficits, including slow growth, multiple limb and organ abnormalities, and intellectual disabilities. Severely affected individuals most often have dominant loss-of-function mutations in the Nipped-B-Like (NIPBL) gene, and milder cases often have missense or in-frame deletion mutations in genes encoding components of the cohesin complex. Previous studies have shown that modest decreases in NIPBL and cohesin activity alter the transcription of many genes that regulate growth and development. We have found that Nipped-B heterozygous mutant Drosophila show reduced learning, memory, sleep, and altered circadian rhythms. These deficits are accompanied by morphological abnormalities in brain structure. These studies confirm that heterozygous Drosophila Nipped-B mutants provide a useful model for understanding CdLS, and provide new insights into the origins of birth defects. We have recently performed RNA sequencing with brain tissue, and are

currently analyzing the data to investigate the global gene expression changes in Nipped-B heterozygous mutant fly brains. This will improve our understanding of affected pathways in patients with CdLS and identify potential drug targets.

Utilizing Gene Expression Signatures to Assess Treatment Strategies For Cornelia de Lange Syndrome Using a Conditional/Invertible Mouse Model of Nipbl Deficiency

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The objective of this research is to determine whether the efficacy of agents, proposed to be potential therapies for treating Cornelia de Lange syndrome (CdLS), can be tested efficiently in mice. The most common underlying genetic defect in CdLS is haploinsufficiency for *Nipbl*, a gene that encodes a protein which is known to both bind cohesin and to have global effects on transcription. We have generated both mouse and zebrafish models of *Nipbl*-deficiency, and have found that *Nipbl*-deficient animals have many features in common with individuals who have CdLS, including small stature, malformation of heart and visceral organs, neurological and behavioral deficits, and limb and craniofacial abnormalities. Moreover, in every tissue tested in these CdLS models, there are small changes in the expression of tens to hundreds of genes, and experiments indicate that it is the synergistic and/or combinatorial action of these many gene expression changes that results in the birth defects observed in CdLS. Thus, we hypothesize that drugs that increase the expression of *Nipbl* may also correct pathological gene expression changes and possibly lead to restoration of function. Unfortunately, *Nipbl*-deficient mice show a high level of perinatal and early postnatal death. To overcome this problem, and also to allow us to test potential therapeutic agents on mice of a controlled genetic background, we have generated a conditional-invertible allelic series of *Nipbl* in mice, which allows us to reduce and restore *Nipbl* function in specific tissues and at specific times during development. We utilized this allelic series to generate mice on a C57Bl6/J inbred background that are also *Nipbl*-deficient solely in the central nervous system (CNS). These mice survive to adult ages, although they have smaller brains than their wild-type littermates. To make it possible to rapidly evaluate potential drug therapies on brain gene expression in these animals, we performed RNA sequencing studies to develop a gene expression signature (GES) that distinguishes the *Nipbl*-deficient brain from normal. We found over 1000 gene expression differences between normal and *Nipbl*-deficient brains; interestingly, gene misexpression was heavily skewed toward pericytes, suggesting that pericyte pathology may play a critical role in the pathology of CdLS. Of genes showing significant changes in expression, 66 genes were identified as fitting specific criteria of fold-change, expression level, and low false-discovery rate. Approximately half were tested and/or validated by Q-RT-PCR on multiple samples

and used to evaluate the brain tissue of *Nipbl*$^{+/-}$ mice (or wild-type controls) that were treated for 4 weeks with a drug shown to increase *Nipbl* expression levels in cell lines and to ameliorate CdLS-like phenotypes in an insect model (*Drosophila*; Dorsett et al. and Lander et al., unpublished observations). Findings from the initial study of drug efficacy, along with results of studies conducted to determine the levels of drug in various organs (using mass spectroscopy) and the effect of the drug on prenatal development, will be discussed.

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Effects of Anti-Oxidant Treatment on Genome Stability and In Vitro Growth of Cornelia de Lange Syndrome Cell Lines

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Cornelia de Lange Syndrome (CdLS), the most common disorder of a class of multi-organ system developmental syndromes termed the cohesinopathies, is caused by mutations in *NIPBL*, *SMC1A*, *SMC3*, *HDAC8*, or *RAD21* genes. CdLS is characterized by dysmorphia, microcephaly, hirsutism, upper limb malformations, cardiac defects, gastroesophageal dysfunction, growth retardation, and intellectual disability. Recently, it has been shown that CdLS cell lines display the downregulation of proteins involved in defence against oxidative stress and consequently an increase in overall oxidative stress. This finding suggests that this phenomenon may directly contribute to several CdLS phenotypic features. In fact, excessive production of reactive oxygen species and the reduction of proteins involved in the response to oxidative stress can cause DNA damage leading to cell death. It is worth noting that *SMC*-mutated probands show evidence of a premature aging process, and manifest several physical changes resulting in a more aged appearance compared to their chronological age. In addition, CdLS cell lines show spontaneous genome instability. Our research investigates the effects of anti-oxidant treatment on both genome stability maintenance and in vitro lifespan of CdLS cell lines.

An Assessment of Medical Care Access, Coordination, and Interactions for Individuals With Cornelia de Lange Syndrome

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Cornelia de Lange syndrome (CdLS) is characterized by many health problems that require multidisciplinary care by various medical specialists as well as allied health professionals. Many individuals with CdLS need lifelong assistance from a caregiver to coordinate these services. Much of the current medical literature and recent public policy has focused on individual access to a medical home and preparation for transition to adult providers; a medical home is defined by the American Academy of Pediatrics as an approach to providing comprehensive care in which the primary care team partners with the family/caregiver to assure that the medical and non-medical needs of the individual are met. However, there is a gap in the literature describing the access to a medical home and comprehensive care in specific disorders, such as the group of individuals with CdLS and their families/caregivers. This study aims to assess the landscape of the medical care of as it relates to the specific needs of the individual with CdLS by focusing on the three categories: access to medical care including proximity, scheduling difficulties, and insurance coverage; coordination of care including participation in a medical home, multidisciplinary care and transition to adult providers; and an assessment of families'/caretakers' overall opinions of provider interactions. An online survey has been offered to families/caregivers of individuals with CdLS, ages 13 years or older, via the CdLS Foundation listserve. The results of this study will enable us to provide anticipatory guidance to families/caregivers about how to prepare for and possibly avoid access and coordination barriers of medical care for the individual with CdLS. Knowledge gained from this study will also help physicians and other allied health professionals, such as genetic counselors, better support families/caregivers of newly diagnosed and younger patients with CdLS as they navigate the complicated healthcare system, and empower them to be better advocates for the individual with CdLS.

Facing the Challenges of Treating Cornelia de Lange Syndrome

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Recent scientific advances have begun to make possible the thought of being able to treat not just the symptoms of Cornelia de Lange Syndrome (CdLS), but also the underlying root causes of the syndrome. Whether through medications that may be able to alter gene expression, or targeted genomic editing, treatment protocols are being investigated by developmental biology researchers in animal models for CdLS. The CdLS Foundation must understand the challenges posed by attempting to test and ultimately implement these treatments in persons affected by CdLS.

CdLS faces a variety of challenges common to other rare conditions. Mutations in several genes lead to CdLS, each resulting in slightly different phenotypic outcomes, and thus amplifying the challenge of treating multiple genetic alterations with similar clinical presentations. Thus, treatment for one CdLS gene or specific mutation may not be effective for treating another gene or specific mutation. Performing large, highly powered clinical trials for rare diagnoses is very difficult (due to the logistics of enrolling sufficient participants), but trying to perform those trials on a smaller subset of affected individuals, each with involvement of a different gene, becomes impractical. Additionally, clinical trials of any size are expensive and frequently require funding from multiple sources.

The CdLS Foundation is proactively seeking ways in which to face these challenges and identify ways to overcome them, as well as to ensure that promising treatments will be evaluated appropriately and safely and eventually used to maximize the potential to help as many individuals as possible. The CdLS Foundation has begun to bring together researchers in a variety of fields to help identify these challenges and seek input from experts who have evaluated treatment options in other rare medical conditions. These discussions have been ongoing and initiated long before the need has arisen. It is hoped that the CdLS Foundation will be able to use this information to establish a roadmap that can be used not just for CdLS, but also for other rare genetic conditions.

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