10th Biennial Scientific Virtual Symposium on Cornelia de Lange Syndrome, Cohesin and Related Genes

June 20 - 21

CdLS Foundation
Cornelia de Lange Syndrome Foundation, Inc.

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Anne Calof, PhD (and Presenter)

Presenter:
Julia O'Connor, PhD
Katherine Ellis, PhD
Gholson Lyon, MD, PhD
Natalie Blagowidow, MD
Antonio Musio, PhD
Sarah Raible, MS
Justin Blair, MS
Maninder Kaur, MS
Philip Boone, MD, PhD
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Jessica Mingins
Carol Li, MD
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Disclosure:
David Litwack, PhD - Employee of Eli Lilly

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Learning Objectives:
1. Appreciate further the spectrum of clinical findings in Cornelia de Lange Syndrome (CdLS)
2. Understand more about the protein cohesin and related genes
3. Learn about embryonic factors related to cohesin
4. Appreciate behavioral issues in CdLS, including autism spectrum
5. Understand benefits of collaboration in cohesin and CdLS research

Monday, June 20, 2022
Meeting starts at 1:55 pm EDT

Behavior and Cornelia de Lange Syndrome; Medical Aspects of CdLS and Related Disorder; *SMC1A* LOF Variants

Moderators: Julia O'Connor, Ph.D. and Matthew Deardorff, M.D., Ph.D.

Time	Topic	Speaker/Moderator
2:00- 2:10 PM	Welcome and Introductions	Antonie D. Kline, Bonnie Royster
2:10- 3:35 PM	**Behavior and Cornelia de Lange Syndrome with Panel**	*Moderator: Julia O'Connor, PhD*
2:10 - 2:25 PM	Neuropsychiatric Functioning and Genotype-Phenotype correlation	Beatrice Allegri
2:25 - 2:40 PM	Social Cognitive Skills	Katherine Ellis
2:40 - 2:55 PM	Triggers and Correlates of Anxiety	Jessica Mingins*
2:55 - 3:10 PM	Intolerance of Uncertainty	Kayla Smith*
3:10 - 3:40 PM	*Q & A and Discussion Panel*	Speakers and Moderator
3:40 - 3:55 PM	Break	
3:55 - 4:50 PM	**Medical Aspects of CdLS and Related Disorder**	*Moderator: Matt Deardorff, MD, PhD*
3:55 - 4:15 PM	COVID-19 Impact on CdLS and Behavior	Antonie D. Kline
4:15 - 4:35 PM	Sleep Disturbance in CdLS**	Carol Li*
4:35 - 4:55 PM	Videoconferencing and Phenotyping for KBG Syndrome	Gholson Lyon
4:55 - 5:15 PM	Q & A and Closing Remarks	Antonie D. Kline
5:15 - 5:30 PM	Break	
5:30 - 7:30 PM	Professional Activity	
5:30 - 6:30 PM	Breakout Session: *Research in SMC1A LOF Variants*	
6:30 - 7:30 PM	Clinical Advisory Board Meeting	Kline and CAB and guests

*Student or Trainee
**CdLS Funded Research

Profiles of social cognitive skills in children with Cornelia de Lange syndrome

Katherine Ellis[1], Sarah White[1], Malwina Dziwisz[2], Antonia Hamilton[1], Beth Webster[2], Paridhi Agarwal[1], Christina Griva[1], Beth Jones[2], Tabby Mclachlan[2], Natali Bozhilova[2], Lauren Jenner[2], Jo Moss[2]

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Background: Although individuals with Cornelia de Lange syndrome (CdLS) show high levels of autistic traits, the presentation of these characteristics differ from non-syndromic autism and other genetic syndromes. These differences may be associated with characteristic profiles of social cognitive skills. We developed a battery of non-verbal assessments to evaluate three social cognitive abilities (imitation, gaze following and implicit mentalizing) considered central to successful interaction, and considered areas of difficulty for autistic children without a genetic syndrome.

Method: Children with CdLS and comparison groups of children with fragile X syndrome (FXS), neurotypical (NT) and autistic (AUT) children aged 4-17 years took part in this study. Gaze following was assessed with a passive viewing paradigm, in which videos of a central cue (ball/cartoon face/human face) directed attention towards one of two objects. Implicit mentalizing was assessed with an implicit anticipatory-looking false belief task. Spontaneous gaze patterns were recorded using eye-tracking for both tasks. Imitation was assessed with an overimitation task, in which we observed whether children intentionally imitated an action sequence that is additional and unnecessary to achieving a goal.

Results: *Imitation:* 88% of NT children and 80% of children with FXS overimitated at least once, compared to 64% of children with CdLS and 58% of AUT children. Of those children who overimitated, the NT and AUT children overimitated on more trials than the CdLS and FXS groups (all $p<.01$). *Gaze following:* Children with CdLS and FXS, and AUT children paid less attention to the eye region of all central cues compared to NT children ($p<.01$). Children with CdLS were less likely to follow directional cues in the non-social condition ($p=.02$), AUT and FXS children were just as likely as NT children to follow directional cues to look at the target object in all conditions (all $p>.05$). NT children looked longer at the target object following facial gaze cues ($p<.01$), while autistic children looked longer at the target object following cartoon gaze cues ($p=.02$). Participants with CdlS and FXS responded similarly to all cues ($p>.05$). *Mentalizing:* NT children ($p<.01$) and children with CdLS ($p=.02$) showed more anticipatory-looking towards the target compared to AUT children.

Discussion: Children with CdLS show similarities and differences in patterns of task performance relative to comparison groups. Children with CdLS showed rates of imitation comparable to autistic children, suggesting difficulties in utilising a critical early social learning tool important for the development of social and communicative abilities. Children with CdLS show similar patterns of gaze following compared to AUT children and children with FXS. However, the value attributed to shifts in gaze differs across participant groups and this may differentially impact the development of social-communication skills. Despite these difficulties, children with CdLS show intact implicit mentalizing abilities, indicating that they are able to spontaneously process other's mental states. Differences in social-cognitive profiles will be considered relative to the behavioural phenotype associated with children with CdLS.

Triggers and correlates of anxiety in Cornelia de Lange syndrome

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Background: People with CdLS have high levels of anxiety relative to the general population, with prevalence estimates ranging from 10-60%. However, little research has focused on the specific triggers of anxiety in people with CdLS who speak few or no words. There were three aims of this study. Firstly, to identify the three most reported anxiety triggers in people with CdLS who speak few or no words using the Anxiety Triggers Questionnaire (ATQ). Secondly, to compare frequency of endorsement of the top three triggers to Fragile X Syndrome (FXS) and autism. Finally, to identify group differences between people with CdLS who endorsed each of the three most reported triggers compared to those who did not.

Methods: This is a secondary analysis of data collected as part of an ongoing large-scale questionnaire study focusing on people with intellectual disability who speak few or no words. Parents or caregivers of 25 people with CdLS, 22 people with FXS, and 99 autistic people participated. Caregivers were recruited through an existing database at the University of Birmingham, NHS Trusts, syndrome support groups and Cerebra. Caregivers completed an online battery of questionnaires including the ATQ, the Wessex Questionnaire, the Responses to Uncertainty and Low Environmental Structure (RULES) questionnaire, the Social Communication Questionnaire (SCQ) and the Sensory Profile Questionnaire.

Results: The most reported triggers for anxiety in the CdLS group were crowds (68%), changes to routine (64%), loud or unexpected noises (56%) and sensory sensitivities (56%). Chi-squared tests indicated no significant differences between groups for endorsement of these triggers (ps > .05). Individuals with CdLS who identified crowds as a trigger had scores indicative of greater intolerance of uncertainty and autism characteristics than those who did not identify crowds as a trigger, $t(22)=2.28$, $p=.032$, $t(15)=2.37$, $p=.031$. Those who identified changes to routine as a trigger had scores indicative of greater intolerance of uncertainty and auditory processing difficulties, $t(22)=3.63$, $p=.001$, $t(23)=2.47$, $p=.021$. Those who identified loud or unexpected noises as a trigger for anxiety had significantly higher scores for autism characteristics, $t(15)=3.80$, $p=.002$. Finally, those who identified sensory sensitivities as a trigger scored more highly on measures of intolerance of uncertainty and auditory processing difficulties, $t(22)=2.68$, $p=.014$, $t(23)=2.31$, $p=.030$, $t(15)=3.24$, $p=.005$.

Conclusions: Triggers for anxiety in people with CdLS who speak few or no words did not differ from people with ASD and FXS when measured with the ATQ. The most common triggers and correlates of anxiety in CdLS overlap with those reported in the autism literature. These findings have implications for our understanding of the factors that may give rise to anxiety in CdLS and with replication may provide specific targets for interventions to reduce anxiety.

Intolerance of uncertainty mediates the relationship between autism spectrum disorder and anxiety in Cornelia de Lange syndrome

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People with Cornelia de Lange syndrome (CdLS) often experience co-occurring mental health conditions such as autism spectrum disorder (ASD) and anxiety. Identifying and understanding behavioural risk markers of anxiety in CdLS is essential to earlier and more accurate diagnoses, thus contributing to better long-term outcomes. Recent studies suggest that intolerance of uncertainty (IU) is a risk factor for the development and maintenance of anxiety and that IU mediates the relationship between ASD characteristics and anxiety in autistic people. Given that people with CdLS are likely to experience co-occurring ASD and anxiety, understanding the relationship between ASD, anxiety, and IU is essential for informing both interventions and theoretical models of anxiety for people with CdLS. This study examined the relationship between ASD characteristics, anxiety, and IU in people with CdLS (n = 33, *Mage* = 13.92 years). ASD characteristics and IU were determined by the Social Responsiveness Scale – Second Edition (SRS-2) and the Intolerance of Uncertainty Scale – Parent Version, respectively. Parent-reported anxiety was assessed using the Anxiety Scale for Children-ASD (ASC-ASD) and the Anxiety, Depression and Mood Scale (ADAMS). Hierarchical multiple regression analyses indicated that both ASD characteristics [$p < .002$] and IU [$p < .001$] significantly predicted anxiety scores. Mediation analyses revealed that IU mediated the relationship between ASD characteristics and anxiety in CdLS [$p < .01$], comparable to the relationship seen in autistic people. To our knowledge, this is the first study to investigate the relationship between ASD characteristics, anxiety, and IU in CdLS. The results of this study suggest that IU plays a key role in the presence of anxiety in CdLS, and therefore it is possible that targeting IU as an intervention for anxiety may be beneficial in this population.

Sleep disturbance in Cornelia de Lange syndrome

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Introduction: Existing literature suggests a high prevalence of sleep disturbance in patients with Cornelia de Lange Syndrome (CdLS). This study uses validated sleep surveys and actigraphy to characterize sleep patterns and disturbance in patients with CdLS.

Methods: This was a prospective cohort study in which caregivers of patients with CdLS completed questionnaires: Obstruction Sleep Apnea (OSA)-18 Quality of Life (QOL) survey, Pediatric Daytime Sleepiness Scale (PDSS), Pediatric Sleep Questionnaire (PSQ), the Children's ChronoType Questionnaire (CCTQ). Children with CdLS and unaffected family members wore wrist actimetry sensors for a week to collect rest/activity cycle information. Cutoffs of ≥ 60 (OSA-18) and > 0.33 (PSQ) were used respectively to indicate OSA. For the PDSS, > 15 points was defined as excessive daytime sleepiness. For the CCTQ, the circadian phase preference was recorded. Total sleep time (TST) and sleep latency were compared between individuals with CdLS and family members. Descriptive statistics were used. Unpaired t-test were used to compare PDSS and PSQ scores in patients with OSA-18 scores ≥ 60 and those with scores < 60, as well as to compare actigraphy results between patients with CdLS and unaffected family members.

Results: Mean age of individuals with CdLS was 15.2 (SD 11.3) years and 63.2% were female. There were 58 OSA-18 questionnaires with a mean score of 49.7 (SD 16.5). Fourteen (24.1%) of participants reported a score ≥ 60. There were 47 PSQ surveys with a mean of 0.39 (SD 0.16); 31 (66.0%) had a score > 0.33. There were 49 PDSS surveys with a mean score of 10.2 (SD 6.5). Eight (16.3%) participants had a PDSS score > 15. Nine (15.0%) of participants classified their children as definitely a morning type, 12 (20.0%) as rather a morning type than an evening type, 19 (31.7%) as neither a morning nor an evening type, 17 (28.3%) as rather an evening type than a morning type, and 2 (3.3%) as definitively an evening type. PSQ scores were higher in individuals with OSA-18 scores of ≥ 60 compared to individuals with OSA-18 scores < 60 (0.52 vs. 0.35, $p = 0.002$). PDSS scores were significantly higher in individuals with OSA-18 scores of ≥ 60 compared to individuals with OSA-18 scores < 60 (15 vs. 8.8, $p = 0.004$). Mean TST was 459.9 minutes (SD 194.3) for 15 individuals with CdLS and 369.1 minutes (SD 139.4) for 28 unaffected family members ($p = 0.08$). Mean sleep latency was 1.64 minutes (SD 1.27) for 15 individuals with CdLS and 2.42 minutes (SD 1.57) for 28 unaffected family members ($p = 0.11$).

Conclusion: Compared to the general population, more children with CdLS exhibit OSA-18 and PSQ scores indicative of OSA. Unlike other adolescents, children with CdLS were not overly sleepy during the day and circadian phase preferences in this population were skewed towards the morning. There were no differences in total sleep time for these individuals with CdLS compared to the normative population in their age group. Existing literature suggests that individuals with CdLS may have more sleep disturbance and therefore may benefit from further objective evaluations, such as polysomnography, to investigate the presence of organic sleep disorders.

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Embryonic tumors in Cornelia de Lange syndrome

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A 27-year-old female with Cornelia de Lange syndrome (CdLS), mosaic for a pathogenic variant in *NIPBL,* with a history of atrial septal defect, leg length discrepancy, gastroesophageal reflux disease (GERD), delayed gastric emptying, pre-diabetes, epilepsy, polycystic ovarian syndrome (PCOS), complete bicornuate uterus, and moderate intellectual disability, presented with a pancreatic mass. Following a Whipple procedure, this was found to be a well-differentiated, low-grade pancreatic neuroendocrine tumor (PNET), without extrapancreatic spread. Family history was negative. Two years later she was diagnosed as having endometrial endometrioid carcinoma involving both cervical stromata, and metastatic to the left ovary, but no myoinvasion. She underwent hysterectomy and subsequently bilateral oophorectomy. Mismatch repair gene involvement and other somatic testing were negative. Chemotherapy was declined.

A 29-year-old female with atypical Cornelia de Lange syndrome, with a history of mild GERD, malrotation, posterior pituitary hypertrophy, delayed developmental milestones but mildly involved, and bilateral 5th finger clinodactyly, found to have a variant of uncertain significance in the *EP300* gene, presented with abdominal pain. CT scan revealed bowel malrotation and an ovarian cyst. Exploratory laparotomy included excision of a right ovarian mass with oophorectomy, and correction of the malrotation. Pathology showed that the mass was a cystic teratoma.

While PNETs are rare pancreatic tumors, they are even rarer in younger patients. They have not been known to be associated with CdLS, although can occur in neurofibromatosis 1, tuberous sclerosis complex, MEN1 and von Hippel-Lindau syndrome. PNETs have been associated with other tumors as well, including endometrial and ovarian (Ehehalt et al., 2011). Endometrial carcinoma also has a known association with PCOS, and both have been previously reported with CdLS although at a slightly older age (Tate et al., 2019).

Other embryonal or germ cell tumors, however, have been previously reported in CdLS, including two cases with Wilms tumor (Santoro et al., 2016; Maruiwa et al., 1988), two cases with sacrococcygeal teratomas (Benait et al., 2015; Dundar et al., 2011), a suprasellar germinoma (Sugita et al., 1986), and a choroid plexus papilloma (Chico-Ponce de Leon et al., 2015). Very few other tumors have been noted in CdLS, although adenocarcinomas have been seen in the esophagus and bowel and are likely a direct result of complications of GERD. Genes of the cohesin complex are associated with widespread gene influence and expression, and somatic mutations of these genes have been widely reported in multiple tumors (Cheng H et al., 2020). Disruption of the regulatory enhancer-promotor interactions by mutations within these genes could lead to the development of tumors (Rivas MA et al., 2021), possibly by progenitor cell proliferation early in embryogenesis, and perhaps a higher risk for embryonal tumors. These should be included in the differential when an unexpected mass is identified in an individual with CdLS.

The role of cohesin in genome maintenance in oocytes and early embryonic development

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De novo mutations in *SMC3*, encoding the cohesin subunit SMC3, account for 1-2% of cases of the developmental disorder Cornelia de Lange syndrome, making it important to understand the molecular function of SMC3 during development. SMC3 supports genome function in multiple ways in multiple cell types, but its role in maintaining the genome during early mammalian embryogenesis is unknown. We depleted SMC3 in mouse oocytes to investigate its role during oogenesis and early embryonic development. We discovered that although depletion of SMC3 in oocytes following meiotic S phase leads to infertility, meiosis was not compromised. We provide evidence that infertility can be attributed to *Smc3* acting as a maternal effect gene, with essential functions in protecting the integrity of chromosomes in zygotes. DNA lesions accumulated following S phase in SMC3-deficient zygotes, followed by mitosis with lagging chromosomes, elongated spindles, micronuclei, and arrest at the 2-cell stage. Importantly, embryonic lethality preceded transcriptional activation of the zygotic genome, suggesting the essential function of SMC3 in the zygote lies in preservation of chromosome integrity and transmission, and not gene expression. Remarkably, although centromeric cohesion was defective in zygotes from juvenile mutant females, embryogenesis was successful, in contrast to the infertility observed in adult mutant females. The different fertility outcomes depending on the age of the mutant female suggests this variable should be accounted for when designing experiments and interpreting early embryonic phenotypes. In summary, SMC3 is essential for repair of spontaneous damage associated with DNA replication and subsequent chromosome segregation in zygotes and protects the integrity of the zygotic genome. Overall, our study indicates that SMC3 is a key factor loaded in oocytes for mitotic competence in zygotes, enabling successful reproduction in female mice. We speculate that mutations in human *SMC3* that significantly compromise zygotic genome integrity are not compatible with early embryogenesis, and therefore would not be found in association with Cornelia de Lange syndrome.

Tracing the origins of birth defects in CdLS using single-cell RNA sequencing

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CdLS is characterized by birth defects in multiple tissues and organs. In animal models of the most common form of CdLS, haploinsufficiency for *NIPBL,* numerous, small changes in gene expression occur in every tissue, as do birth defects similar to those in CdLS. Using the *Nipbl*+/- mouse as a model, we documented abnormal heart development as early as cardiac crescent (CC) stage—the time of initial coalescence of heart progenitors after gastrulation—which suggests that the cause of heart defects in CdLS may occur as early as gastrulation. To investigate this, we performed single-cell RNA sequencing on both CC- and gastrulation-stage wildtype and *Nipbl*+/- mouse embryos. Interestingly, we found that *Nipbl*+/-embryos overexpressed *Nanog* at both stages. *Nanog* encodes a transcriptional repressor involved in maintaining pluripotency and is normally *transiently* expressed first in pre-implantation embryos, and then later during gastrulation. In *Nipbl*$^{+/-}$ mice, we observed that *Nanog* remained elevated post-gastrulation, along with misexpression of developmentally-significant genes known to be targets of *Nanog*, including genes associated with pluripotency (*Pou5f1*/Oct4), left-right patterning (*Tdgf1*, *Lefty2*, *Nodal*), anterior-posterior patterning (*Hox* genes), and primitive erythropoiesis (*Tal1*, *Lmo2*, *Hbb-bh1*). Accompanying these changes were changes to the allocation of cells to *Mesp1*-expressing cardiac progenitors, the first and second heart fields, and rostral neural crest (which gives rise to craniofacial structures). These results suggest that a failure to downregulate *Nanog* expression after gastrulation, and the transcriptional dysregulation that ensues, lead to misallocation and/or dysfunction of early progenitor cell populations. Current work is focused on determining how these observed phenomena may give rise to the heart and craniofacial defects of CdLS.

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Genome instability is a marker of Cornelia de Lange syndrome cells

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Cornelia de Lange syndrome (CdLS) is a rare developmental disorder with an incidence of between 1:10,000 and 1:30,000 live births. Common characteristics of CdLS include cognitive impairment, pre- and postnatal growth retardation, microcephaly, facial dysmorphia, hirsutism, and upper extremity defects. CdLS is caused by mutations in *HDAC8*, *NIPBL*, *RAD21*, *SMC1A* and *SMC3* genes belonging to the cohesin-core or its regulators. Recently, we showed that two CdLS patients carrying a mutation in *SMC1A* gene are characterized by reduced cell life span, high level of oxidative stress and genome instability. Up until now, no systematic study has been performed to investigate whether genome instability is a marker of CdLS patients. To gain insight into this topic, we cultured CdLS cell lines harboring mutations in *SMC3*, *NIPBL* and *HDAC8* genes. We found that CdLS cells became senescent around the 25th passage with a considerable decrease in their in vitro lifespan compared with control cell lines. This senescence was confirmed with a □-galactosidase assay. Next, we analyzed the level of oxidative stress during cell progression through in vitro culture. To study global oxidative stress, we measured the level of protein carbonyls by ELISA. At early passage, the protein carbonyl content in CdLS cells was significantly higher than control cells. In addition, the frequency of spontaneous chromosome aberrations was also found to be significantly higher in all-mutated cell lines. These results indicate that genome instability may be considered a specific marker of CdLS.

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The Multidisciplinary Clinic for Individualized Management of Cornelia de Lange Syndrome at The Children's Hospital of Philadelphia: 10 years of growth and discovery

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Cornelia de Lange syndrome (CdLS) is a multisystem developmental diagnosis with variable growth, cognitive, craniofacial, limb, intestinal, cardiac, and other systemic differences. Given the clinical complexities of CdLS and the need for multispecialty care, the Center for Cornelia de Lange Syndrome and Related Diagnoses was established at The Children's Hospital of Philadelphia (CHOP). This Center was developed to provide a comprehensive and integrated approach to clinical management across the lifespan and to drive clinical and basic research elevant to individuals with CdLS and related diagnoses. The multidisciplinary clinic of the Center functions under the hypothesis that by understanding the clinical issues in CdLS and training experts in relevant specialties to proactively manage them, we will be able to improve the quality of life and cognitive/behavioral outcomes of our patients. Over the past 10 years our clinical program has evaluated over 400 patients from across the US and the world in our monthly multidisciplinary clinics. Since its inception the subspecialties involved in this clinic have expanded beyond the initial core involvement of genetics, gastroenterology, physical therapy and child development to also include dentistry and occupational therapy. The clinic's ongoing collaboration with the National CdLS Foundation has allowed for additional support and education serving as a valuable resource for the families attending the Center. The multidisciplinary clinic also serves as an interface with the Center's research goals that aims to improve medical management and scientific understanding of CdLS. With advancing technology, rapid growth in gene discovery has contributed to the characterization of CdLS-like diagnoses which phenotypically overlap with CdLS, including CHOPS syndrome (due to mutations in the *AFF4* gene), as well as novel diagnoses in previously described CdLS genes, such as SMC1A-related epilepsy and neurodevelopmental disorder. Overall, our Center provides a setting in which individuals with CdLS and related diagnoses can receive coordinated care, comprehensive services, family support, and the opportunity to participate in translational research. In addition, the Clinic models applicability to other neurodevelopmental diagnoses which has readily allowed for the initiation of a Pallister-Killian Syndrome (PKS) and Kabuki syndrome multidisciplinary clinic adapted from the CdLS Center's clinical operations. This presentation will provide an overview of the Center structure at CHOP and it's evolution over the past 10 years, our experience of applying a multidisciplinary integrated clinical and research approach to the management of over 400 patients with CdLS, select related diagnoses that have been described from this patient population, and the application of this model clinical setting to other multisystem developmental diagnoses.

Return of individual research results from the genomic diagnostics in Cornelia de Lange syndrome, related diagnoses, and structural birth defects study: a retrospective experience

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Background: Cornelia de Lange syndrome (CdLS) is a rare multisystem genetic diagnosis with an estimated prevalence of 1/10,000 live births. The majority of cases are caused by variants within genes having a structural or regulatory function in the cohesin complex. However, over 30% of individuals with a clinical diagnosis of CdLS and concurrent genetic testing remain without a molecular diagnosis. The Genomic Diagnostics in Cornelia de Lange Syndrome, Related Diagnoses, and Structural Birth Defects Study recently completed whole genome sequencing of 400 individuals from around the world with clinically suspected CdLS, or a related diagnosis, and their family members. The CdLS research program in which these families had been enrolled at the Children's Hospital of Philadelphia (CHOP) is a historical project that has been ongoing for over 25 years and clinicians, investigators, and enrolled families have long awaited these findings. Initial iterations of the research protocol did not include a mandate to return individual results; however, bioethics suggest there may be an ethical obligation to do so. This study provides a retrospective experience of returning whole genome sequencing research results to families from a large rare disease biorepository.

Methods: Genome sequencing of 96 trios, 30 duos, and 52 singletons was performed. For those with a likely molecular etiology identified, recontact was initiated via the contact information on file. Respondents were invited and scheduled for a meeting to disclose results followed by a short interview survey. The survey interviews were aimed at understanding families' impressions and impact of their child's molecular diagnosis, attitudes towards the return of individual research results, and perceived utility of this study and genetic testing in general.

Preliminary data & proposed analysis: Pathogenic and likely pathogenic results were identified in 53 probands, giving a diagnostic yield of 30% (53/178). 51% (27/53) of individuals had a variant identified in a gene other than one of the 5 known CdLS genes. No contact information was available for 4 individuals. Five individuals were not contacted as they had previously received a molecular diagnosis through the CHOP clinical team, or had contacted researchers with their clinical results prior to this study. Contact was initiated for 44 individuals with a response rate of 77% (34/44) at present. We were informed that 38% (13/34) of individuals were already aware of these results from clinical testing performed in the interval between enrollment and identification of a result through the research study. Therefore, to our knowledge, 34% (18/53) individuals were aware of their molecular diagnosis by the time of this study. Ten interviews have been completed and transcript analysis is ongoing. Seven of the 10 individuals interviewed were unaware of a molecular diagnosis at the time of recontact. Interviewees all expressed positive sentiments to our recontact after so many years. Nine of 10 interviewees had a variant identified in a gene other than one of the 5 known CdLS genes, many of which expressed they had at one time questioned whether CdLS was the correct clinical diagnosis for their child. However, majority stated they had long identified with the CdLS clinical diagnosis, are active members of the CdLS Foundation, and plan to remain members and identify with the CdLS clinical diagnosis. Barriers to recontact and interview responses will be further analyzed and presented.

Genomic analyses in Cornelia de Lange syndrome and related diagnoses: genetic heterogeneity, genotype-phenotype correlations and common mechanisms

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Background: Cornelia de Lange Syndrome (CdLS) is a rare, dominantly inherited multisystem developmental diagnosis characterized by variably expressed manifestations of growth and developmental delays, upper limb involvement, hypertrichosis, cardiac, gastrointestinal, craniofacial and other systemic features. Pathogenic variants in genes encoding cohesin complex structural subunits and regulatory proteins (NIPBL, SMC1A, SMC3, HDAC8, and RAD21) are the major pathogenic contributors. Mutations in additional genes such as *ANKRD11*, *EP300*, *AFF4*, *TAF1*, *BRD4 and others,* have also been shown to result in a CdLS-like phenotype. The common role that these genes, and others, play as critical regulators of developmental transcriptional control has led to the group of diagnoses caused by disruption of these genes being referred to as disorders of transcriptional regulation (or "DTRs").

Methods: A variety of screening methods have been used over the past 20+ years to gain insight into this cohort of CdLS patients, the rapid evolution of sequencing technology has allowed for re-examination of previously mutation negative individuals and for novel insights into genetic causes of this syndrome. The mutation discovery methods employed in this study include targeted CdLS NGS panels, arrays, whole exome sequencing and more recently whole genome sequencing. Genomic analysis was conducted using GATK Broad best practices workflows aligned to human reference genome hg38, variants were annotated using ANNOVAR and SnpEff, population frequency cutoffs using gnomAD.

Results/Data: Here, we report the results of a comprehensive molecular analysis in a cohort of 714 probands with typical and atypical CdLS in order to delineate the genetic contribution of mutations in cohesin and related proteins, genotype-phenotype correlations and the utility of genome sequencing in understanding the mutational landscape in this population. Pathogenic mutations were identified in 414 (58%) probands: *NIPBL* (66%), *SMC1A* (9%), *HDAC8* (6%), *SMC3* (4%), *RAD21* (1%), other causative genes (16%). Rare CNVs not encompassing known CdLS Loci were identified in 2.7%. Genome sequencing was performed on 178 CdLS probands for whom targeted CdLS gene mutational analyses failed to identify a pathogenic cause. A causative mutation was identified in 31%. In 16% mutations in known cohesin genes were identified, 15% had mutations identified in known disease-causing genes associated with other diagnoses that either overlap or phenocopy the CdLS phenotype (*AFF4*, *ANKRD11*, *ARCN1*, *ARID1B*, *ASXL2*, *ASXL3*, *BRD4*, *CERT1*, *CHD2*, *EP300*, *KCNH1*, *KMT2A*, *PACS1*, *PHF6*, *SETD5*, *SMARCA2*. *SMARCA4*, *SOX11*, *STAG2*, *TAF1*, *USP7*). In addition novel CdLS candidate genes were identified (*NAALADL2*, *PDS55A1*, *ATL1*, *HERC5*, *ITGB8*, *PHRF1*, *BSN*).

An old new gene for Cornelia de Lange syndrome: *NAALADL2*

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Cornelia de Lange Syndrome (CdLS) is a rare, dominantly inherited multisystem developmental diagnosis characterized by highly variable manifestations of growth and developmental delays, upper limb involvement, hypertrichosis, cardiac, gastrointestinal, craniofacial and other systemic features. Pathogenic variants in genes encoding cohesin complex structural subunits and regulatory proteins (*NIPBL*, *SMC1A*, *SMC3*, *HDAC8*, and *RAD21*) are the major pathogenic contributors to CdLS. Heterozygous or hemizygous mutations in these five genes have been found to be contributory to CdLS, with mutations in the *NIPBL* gene accounting for the majority (over 60% of CdLS cases), and the only gene identified to date that results in the severe or classical form of CdLS when mutated. Although great progress has been made in identifying the genetic causes of CdLS, there remains a significant subset (~30%) of affected individuals with no identifiable pathogenic variant, suggesting that there are additional genes that have yet to be discovered. To identify additional potential causal novel disease loci and new disease genes for CdLS, as part of our genome sequence project we performed WGS on 178 'mutation-negative' probands with typical and atypical features of CdLS who were not found to have a causative mutation in one of the known CdLS genes on more conventional analyses. 59(33%) causative mutations were identified in total, of which 22(37%) were in known CdLS genes. In addition, a series of disease-contributing variants were identified in 37 additional genes, 30 (~51%) of which cause distinct but overlapping/phenocopying diagnoses, (*ANKRD11(5)*, *ARCN1(1)*, *ARID1B(3)*, *ASXL2(1)*, *ASXL3(1)*, *CERT1(1)*, *EP300(2)*, *KCNH1(2)*, *KMT2A(2)*, *PACS1(1)*, *PHF6(1)*, *SETD5(3)*, *SMARCA2(1)*. *SMARCA4(2)*, *SOX11(1)*, *STAG2(1)*, *TAF1(1)*, *USP7(1))*, that share common underlying pathogenic molecular mechanisms. Amongst this cohort we identified 2 probands with variants in the *NAALADL2* (N-acetylated alpha-linked acidic dipeptidase-like 2) gene. *NAALADL2* was identified at the 3q26.3 breakpoint in a child with CdLS that had a de novo balanced translocation [t(3;17)(q26.3;q23.1)] reported by Ireland et al, 1991and characterized by Tonkin et al. in 2004. Mutational screening of this gene at the time in a cohort of CdLS individuals failed to identify any mutations. The 3q26.3 region was of interest as a possible CdLS locus due to the phenotypic overlap of individuals with the 3q26 duplication syndrome and CdLS, suggesting that a gene within this region may result in CdLS when mutated or deleted. Identified mutations in our cohort include one *de novo* missense c.511A>C, p.Thr171Pro and a nonsense mutation of unknown inheritance (proband only sample) c.2098A>T, p.Arg700*, in probands with similar mild-moderate phenotypes. Preliminary data indicates that the NAALDL2 protein co-IPs with the NIPBL protein (K. Shirahige personal communication).

WAPL loss is associated with neurodevelopmental phenotypes, suggesting a cohesin balance disorder

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Cohesin orchestrates 3D genome organization and gene expression programs via DNA loop extrusion to form topologically associating domains (TADs). Loss of cohesin or its positive regulators (e.g. the cohesin loader NIPBL) causes prominent neurodevelopmental phenotypes including Cornelia de Lange syndrome (CdLS). Via an analysis of copy-number alterations in >900,000 individuals, we found that autosomal cohesin genes have elevated predicted haploinsufficiency (pHI) and triplosensitivity (pTS) scores (mean rank pHI 99.2%ile, pTS 96.2%ile of 17,263 genes), suggesting that a disturbance of cohesin balance in either direction is pathogenic. Thus, we hypothesized that loss of the cohesin releaser WAPL, which serves an opposing function to NIPBL, would cause a novel disorder. We sought and identified 15 cases of heterozygous *de novo WAPL* variants, including missense and truncating changes, in children and adults. Developmental delay of mild-moderate severity is ubiquitous in this case series, and some birth defects (e.g. club foot) may be enriched but are pending further phenotypic analysis. To further probe the effect of *WAPL* haploinsufficiency on neurodevelopment, we performed a gene-centric burden analysis of exome sequencing data from >30,000 individuals with developmental delay. *WAPL* variants are enriched in these cases ($q<0.05$), not only adding statistical confirmation to our subject data, but also nominating *WAPL* (along with *BMPR1A*) as one of two candidate driver genes for neurodevelopmental phenotypes in the recurrent 10q22q23 deletion syndrome. Finally, we CRISPR-engineered >50 cell lines with *WAPL* LoF, *NIPBL* LoF, or 10q22q23 deletions for analysis via ongoing functional genetic methods to assay the presence and consequence of perturbed cohesin balance in these disease models.

Panel

Understanding CdLS through Collaboration
A panel discussion of where we stand and where we are going with therapeutic interventions for CdLS

Moderators: Anne L. Calof, PhD, University of California, Irvine, CA
Lynne Kerr, MD, PhD, University of Utah, Salt Lake City, UT

Panel Participants: Sarah Raible, MS, University of Pennsylvania, Philadelphia, PA
Ian Krantz, MD, University of Pennsylvania, Philadelphia, PA
Arthur Lander, MD, PhD, University of California, Irvine, CA
David Litwack, PhD, Prevail Therapeutics, New York, NY
Rich Haaland, PhD, CdLS Foundation, Avon, CT

To date, there are no accepted therapeutic interventions that would attempt to correct pathologic changes in Cornelia de Lange Syndrome (CdLS) at their root cause: the disruption of genes that encode proteins of the cohesin complex, cohesin-associated proteins, and enzymes that affect epigenetic modifications of DNA. Instead, clinical intervention and preventative care remain the mainstay of managing children and adults with CdLS. Why is this the case, and what are the issues associated with global therapeutic interventions for such a complex syndrome? In this panel, clinicians and basic scientists will come together to discuss these issues.

The questions that will be addressed include:

1. What do we know about families' interest in therapies?
2. What approaches are available for us to develop therapies in model systems (animals, cell lines)?
3. What has been done so far?
4. What is on the horizon, through the efforts of the Foundation and others?
5. If we had a candidate therapy that showed promise in a model system, what would it take to be able to get a clinical trial approved: Clinical Endpoints? Study population? Feasible Timeline?
6. How does the path to therapeutic intervention differ between pharmacological therapy and gene therapy?

Panel

Time	*Topic*	*Speaker/Moderator*
3:10 – 3:15pm	Introduction of Panel title, moderators and participants	Calof, Kerr
3:15 – 3:25pm	Current status of clinical CdLS therapies/input from families	Krantz, Raible
3:25 – 3:35pm	The FDA clinical trial approval process: issues and endpoints	Litwack
3:35 – 3:45pm	Complexities of pharmacological and gene therapy for CdLS	Lander
3:45 – 4:25pm	Discussion: panel and audience participation	Haaland, Calof, Kerr
4:30pm	**End of Symposium**	

Speaker	Title	Degree	Institution	Location
Antonie Kline akline@gbmc.org	Director, Clinical Genetics	M.D	Greater Baltimore Medical Center	Baltimore, MD
	Medical Director		CdLS Foundation	Avon, CT
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Jessica Mingus* 180146152@aston.ac.uk			Aston University	Birmingham, UK
Kayla Smith* Kayla.smith@warwick.ac.uk			Warwick Medical School, University of Warwick	Coventry, UK
Matt Deardorff mdeardorff@chla.usc.edu	Professor of Pathology, Principal Investigator and Director of Children's Hospital of Los Angeles' Personalized Care Program	M.D., Ph.D.	Children's Hospital of Los Angeles'	Los Angeles, CA
Carol Li* Carol.li@cchmc.org	Clinical Fellow	M.D.	Cincinnati Children's Hospital Medical Center	Cincinnati, OH
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Anne Calof alcalof@uci.edu	Professor, Anatomy & Neurobiology, Cell & Developmental Disorders	Ph.D.	University of California	Irvine, CA
Lynne Kerr Lynne.kerr@hsc.utah.edu		M.D., Ph.D.	Health Sciences Center University of Utah	Salt Lake City, UT
David Litwack Litwack.david@lilly.com	Senior Director, Scientific Strategy	Ph.D.	Prevail Therapeutics, Eli Lilly	New York, NY
Arthur Lander adlander@uci.edu	Professor, Developmental and Cell Biology	M.D., Ph.D.	University of California	Irvine, CA

*Trainee; **Recipient of CdLS Foundation Award