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ORIGINAL INVESTIGATION

Mutations in chromatin regulators functionally link Cornelia de Lange syndrome and clinically overlapping phenotypes

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Abstract The coordinated tissue-specific regulation of gene expression is essential for the proper development of all organisms. Mutations in multiple transcriptional regulators cause a group of neurodevelopmental disorders termed "transcriptomopathies" that share core phenotypical features including growth retardation, developmental delay, intellectual disability and facial dysmorphism. Cornelia de Lange syndrome (CdLS) belongs to this class of disorders and is caused by mutations in different subunits or regulators of the cohesin complex. Herein, we report on the clinical and molecular characterization of seven patients with features overlapping with CdLS who were found to carry mutations in chromatin regulators previously associated to other neurodevelopmental disorders that are frequently considered in the differential diagnosis of CdLS. The identified mutations affect the methyltransferase-encoding genes *KMT2A* and *SETD5* and different subunits of the SWI/SNF chromatin-remodeling complex. Complementary to this, a patient with Coffin–Siris syndrome was found to carry a missense substitution in *NIPBL*. Our findings indicate that mutations in a variety of chromatin-associated factors result in overlapping clinical phenotypes, underscoring the genetic heterogeneity that should be considered when assessing the clinical and molecular diagnosis of neurodevelopmental syndromes. It is clear that emerging molecular

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mechanisms of chromatin dysregulation are central to understanding the pathogenesis of these clinically overlapping genetic disorders.

Introduction

Temporospatial regulation of gene expression is critical for proper functioning of all cellular processes. At any given time, thousands of genes need to be activated or repressed in a precise and coordinated manner. To achieve this, multiple mechanisms are employed by cells, including: (1) the recruitment of *trans*- and *cis*-acting elements; (2) the modification of DNA accessibility by chromatin remodeling complexes and histone modifiers and (3) the recruitment or mobilization of the RNA polymerase II machinery (Tang et al. 2010).

Over recent years, mutations in multiple transcriptional regulators have been linked to a growing spectrum of neurodevelopmental disorders, recently grouped into the emerging category of so-called "transcriptomopathies" (Yuan et al. 2015; Izumi 2016). Patients with these syndromes share a number of clinical features including growth retardation, intellectual disability, developmental delay and a combination of similar facial features. Cornelia de Lange syndrome (CdLS, OMIM #122470, 300590, 610759, 300882, and 614701) is a rare congenital multisystem disorder that falls within this category. To date, mutations in five different cohesin-related genes, *NIPBL*, *SMC1A*, *SMC3*, *RAD21* and *HDAC8,* have been described to cause several subsets of CdLS, but further locus heterogeneity is predicted (Krantz et al. 2004; Tonkin et al. 2004; Musio et al. 2006; Deardorff et al. 2007, 2012a, b). The cohesin complex is well known for its role in mediating sister chromatid cohesion and DNA double-strand break repair (Mehta et al. 2013). However, in the last decade, extensive analysis of the function and structure of cohesin has revealed that the complex is involved in many other chromatin-related processes, which include transcriptional regulation, chromatin remodeling, chromosome condensation and morphogenesis (Mehta et al. 2013). Consistent with these other key functions, cell lines from patients with CdLS do not display sister chromatid cohesion defects (Castronovo et al. 2009). This underscores that the etiopathology of CdLS and related disorders is not directly linked to the disruption of sister chromatid cohesion but to the altered ability of the cohesin complex to mediate other more dosage-sensitive cellular functions such as transcriptional regulation (Liu and Krantz 2009).

Consistent with a central role of transcriptional dysregulation in patients with CdLS or CdLS-overlapping phenotypes, mutations in chromatin-associated factors other than cohesin have recently been described. These include alterations in the transcriptional regulator genes *EP300*, *AFF4*, *ANKRD11* and *KMT2A*, which have been previously associated with other multisystem developmental disorders that are frequently considered in the differential diagnosis of CdLS (Ansari et al. 2014; Woods et al. 2014; Izumi et al. 2015; Yuan et al. 2015; Parenti et al. 2016a). Mutations in *EP300*, encoding an acetyltransferase, cause Rubinstein–Taybi syndrome type 2 (RSTS2, OMIM #613684), mutations in the transcriptional repressor *ANKRD11* lead to KBG syndrome (KBGS, OMIM #148050) and mutations in the histone methyltransferase *KMT2A* cause Wiedemann–Steiner syndrome (WDSTS, OMIM #605130). Very recently, alterations of *AFF4*, encoding a core component of the super elongation complex, have been described as the cause of a new CdLS-overlapping syndrome (CHOPS, OMIM #616368) (Roelfsema et al. 2005; Sirmaci et al. 2011; Jones et al. 2012; Izumi et al. 2015). Notably, all of these disorders demonstrate a significant clinical overlap with CdLS, each presenting with shared clinical features that include growth retardation, intellectual disability, developmental delay and facial dysmorphisms such as synophrys, a short nose with anteverted nares, a smooth philtrum and a thin upper lip. In addition to the identification of mutations in transcriptional regulatory genes that can lead to a CdLS-overlapping phenotype, mutations in "CdLS" genes can underlie clinical phenotypes attributed to mutations in other chromatin regulators. For example, Coffin–Siris syndrome (CSS, OMIM #135900) and Nicolaides–Baraitser syndrome (NCBRS, OMIM #601358), which themselves demonstrate some clinical overlap with CdLS, are caused by mutations in different subunits of the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex,

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that shows some shared functions with cohesin (Santen et al. 2012; Tsurusaki et al. 2012). Further strengthening this connection, a direct functional interaction between the cohesin loader NIPBL and the SWI/SNF complex in yeast has been recently demonstrated. Particularly, Scc2, the yeast orthologue of NIPBL, is recruited by the SWI/SNF complex to broad nucleosome-free regions, which both subsequently help to maintain (Lopez-Serra et al. 2014). These findings directly indicate the combined roles of cohesin and SWI/SNF in regulating the expression of common target genes and/or genomic regulatory elements.

To further elucidate this important interaction in human development, here, we report on the clinical and molecular characterization of seven patients presenting with features overlapping CdLS, for whom the analysis of the five known CdLS-genes did not identify any disease-causing mutation. Subsequent use of multiple next-generation sequencing technologies identified mutations in key chromatin-associated factors genetically different from cohesin. Mutations in the methyltransferases KMT2A or SETD5 were identified in three patients, and two different subunits of the chromatin-remodeler SWI/SNF complex were mutated in four. To complement this, eight unsolved patients with a clinical diagnosis of CSS/NCBRS were investigated for the presence of mutations in the known CdLS genes. This led to the identification of a missense substitution in *NIPBL* in one patient with CSS, who was previously described by Wieczorek and colleagues in 2013, attesting that interrelated genes underpin closely similar phenotypes.

In summary, our findings suggest that mutations in different but functionally related chromatin-associated factors might result in strongly overlapping clinical pictures. As the primary role of these factors concerns chromatin, and only indirectly regulation of transcription, we propose that these conditions could be grouped within the category of "chromatin dysregulation disorders" rather than "transcriptomopathies" per se. Also, these findings underscore the necessity to further identify and molecularly characterize the disease-relevant mechanisms involved in the regulation of chromatin function both in normal and pathological backgrounds. Not only will this be of utmost importance for making accurate clinical and molecular diagnoses, but also will it be critical in understanding the pathogenesis and developing therapies that may serve to improve a wider range of disorders caused by disruptions in chromatin regulation.

Materials and methods

Patients

All patients were recruited as part of an international collaboration between investigators in Germany, Austria, Spain, Turkey and Colombia. The study was performed according to the Declaration of Helsinki protocols and was approved by the local institutional review board (ethical votum 12-5089-BO for CRANIRARE and 08-3663 forM-RNET). Informed consent was obtained from all individuals and/or their legal guardians included in this study. An additional consent was obtained for the publication of photographic material identifying patients. All children were born to healthy parents and underwent complete physical and dysmorphology evaluation by their referring clinical geneticists. A list of clinical features observed in each patient is provided in Table 1.

DNA isolation

Genomic DNA was isolated from blood or buccal mucosa. Extraction of the genomic material was performed with the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) or with the Gentra Puregene Buccal Cell Kit (Qiagen) according to the manufacturer's instruction.

Targeted next-generation sequencing

Targeted next-generation sequencing was based on the design of a custom-made gene panel for the Personal Genome Machine (Ion Torrent PGM) with the AmpliSeq Designer Tool (Life Technologies, Darmstadt, Germany) as previously described (Braunholz et al. 2015).

Exome sequencing

Targeted enrichment was performed using the SureSelect XT Human All Exon 50 Mb kit, versions 4 or 5 (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed on HiSeq 2500 systems (Illumina, San Diego, CA, USA). Read tags were aligned with the human reference genome hg19 (GRCh37) using Burrows—Wheeler Aligner version 0.7.5 (Li and Durbin 2010). Variant calling was performed using SAMtools version 0.1.18, PINDEL version 0.2.4t and ExomeDepth version 1.0.0. Subsequently, variants were filtered using the SAMtools varFilter script. Shortly, variants that were predicted to impact the coding sequences and with a frequency in the population lower than 2% were retained. Resulting variants were filtered according to a dominant/de novo inheritance model.

Sanger sequencing

PCR products were sequenced with the Big Dye® terminator v3.1 Sequencing Kit and run on the ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Table 1 Clinical features of the reported patients

Patient number	1	2	3	4	5	6	7	8			
Country of origin	Turkey	Turkey	Germany/India	Germany	Colombia	Germany	Spain	Turkey			
Gender	m	m	f	f	f	m	m	f			
Gene	*KMT2A*	*SETD5*	*SETD5*	*SMARCB1*	*ARID1B*	*ARID1B*	*ARID1B*	*NIPBL*			
RefSeq	NM_001197104	NM_001080517		NM_001317946	NM_020732	NM_020732		NM_133433			
Exon	27	16		7	9	12		40			
DNA change	c.8590C > T	c.2212_2213delAT	del(3)(p25.3)	c.998A > G	c.2692C > T	c.3136-2A > G	del(6)(q25.3q26)	c.6886A > G			
Protein change	p.(Gln2864*)	p.(Met738Valfs*27)		p.(Lys333Arg)	p.(Arg898*)	p.(Lys1046Leufs*18)		p.(Ser2296Gly)			
Origin	de novo	de novo	Father not available	Father not available	de novo	de novo	de novo	de novo			
Gestational weeks	35 weeks	38 weeks	41 weeks	39 weeks	39 + 5	40 weeks	39 weeks	42			
Length cm/SD	45/−1.04	52/0.39	51/−0.55	45/−2.77	49/−1.15	51/−0.65	43.5/−3.65	na			
Weight g/SD	2720/0.24	3670/0.83	2850/−1.7	2370/−2.33	3220/−0.51	3270/−0.8	2270/−2.81	3300/−0.75			
OFC cm/SD	34/0.56	36/0.79	34/−0.92	30.5/−3.15	na	32/−2.77	31.5/−2.92	na			
Age	15 years	7 years	6 years 5 months	20 years	2 years	5 years 4 months	10 years	11 years			
Height cm/SD	155.6/−2.12	123/−0.26	113/−1.53	136/−5.32	80/−1.93	105/−1.84	126.6/−2.23	135/−1.67			
Weight kg/SD	49.7/−1.06	21/−0.98	na	32.7/−5.51	9/−2.24	17.2/−1.14	28.3/−1.08	na			
OFC cm/SD	53.5/−1.62 SD	52/−0.44	50/−1.05	na	47.5/−0.62	52/0.14	50/−2.69	52/−0.85	CdLS	CSS	WDSTS
Brachycephaly	−	−	−	na	+	+	−	na	+	−	−
Microcephaly	+	−	−	+	−	−	+	−	+	+	+
Low anterior hairline	+	+	−	+	+	−	+	+	+	+	+
Sparse scalp hair	−	−	−	+	−	+	−	−	−	+	−
Flat facies	−	−	−	−	−	−	−	−	−	−	+
Coarse facies	−	−	−	−	−	−	−	+	−	+	−
Frontal bossing	−	−	−	+	−	−	+	+	−	−	−
Arched eyebrows	+	+	−	+	+	+	−	−	+	+	−
Thick eyebrows	+	+	+	+	+	−	+	+	+	+	+
Synophrys	+	+	−	+	+	+	+	+	+	±	+
Long eyelashes	+	−	+	+	+	−	+	na	+	+	+
Visual problems	−	−	−	+	+	−	−	na	+	+	+

Table 1 continued

Patient number	1	2	3	4	5	6	7	8			
Depressed nasal bridge	+	−	+	+	+	−	−	+	+	+	−
Anteverted nostrils	−	−	−	−	+	−	+	+	+	+	−
Thick alae nasi	+	−	−	+	+	−	+	+	−	+	+
Broad nasal tip	+	−	+	+	+	−	+	+	−	+	+
Long philtrum	+	−	−	+	+	+	−	+	+	+	+
Smooth philtrum	+	+	+	−	−	+	+	+	+	−	−
Large mouth	−	−	−	−	−	−	+	+	−	+	−
Downturned corners of the mouth	−	−	−	+	−	−	−	−	+	+	±
Thin upper vermilion	+	−	+	+	+	+	+	+	+	+	+
Thick lower vermilion	−	−	−	−	+	−	+	+	−	+	−
Delayed dentition	na	−	na	na	+	−	−	na	−	+	−
Hypertrichosis	+	−	−	na	+	−	+	+	+	+	+
Hairy elbows	+	−	−	na	−	−	−	−	−	−	+
Small hands/feet	+	−	−	+	+	−	−	+	+	−	+
Brachydactyly	−	−	−	+	−	−	+	na	+	−	+
Proximally set thumbs		−	−	na	+	−	+	+	+	−	−
Clinodactyly 5th finger	+	−	−	+	+	−	+	+	+	−	+
Short 5th finger	−	−	−	na	+	−	+	+	+	+	+
A/Hypoplasia of the distal phalanges of the fifth finger	−	−	−	−	−	−	+	+	−	+	−
A/Hypoplastic nails	−	−	−	+	−	−	+	+	−	+	−
Syndactyly of toes	+	−	−	+	−	−	+	−	+	−	+
Prominent interphalangeal joints	−	−	−	na	−	−	+	na	−	+	−

Table 1 continued

Patient number	1	2	3	4	5	6	7	8			
Prominent distal phalanges	–	–	–	na	–	–	+	na	–	+	–
Joint laxity	na	–	–	na	+	–	+	na	–	+	–
Delayed bon age	–	na	na	na	na	na	–	+	–	+	–
Cardiac anomalies	–	–	–	+	+	–	+	na	+	±	±
Genitourinary anomalies	–	–	–	na	–	–	+	na	+	±	±
CNS anomalies	+	–	–	na	+	–	–	–	±	+	±
Feeding problems	–	–	+	+	+	–	+	na	+	+	+
Hearing problems	–	–	–	+	–	–	+	+	+	+	+
Seizures	–	–	–	–	–	–	–	+	+	+	
Frequent infections	–	+	–	–	+	–	–	na	–	+	–
ID	(+)	(+)	+	+	+	+	+	++	+	+	+
Behavioral problems	+	–	+	–	–	+	+	Autism	+	+	+
Age of sitting independently	36 months	na	24 months	na	7 months	na	9 months	na	Delayed	Delayed	Delayed
Age of walking independently	36 months	16 months	36 months	30 months	24 months	22 months	30 months	48 months	Delayed	Delayed	Delayed
Age of first words	30 months	18 months	5 years	9–12 months	Delayed	30 months	10 months	30 months	Delayed	Delayed	Delayed

na data not available

Electropherograms were analyzed with ChromasPro Software version 1.7.6 (Technelysium Pty Ltd., Tewantin QLD, Australia) and aligned with the wild-type sequences of the respective genes. Electropherograms of patients with point mutations are available as Online Resource 1.

CGH-array

Genomic DNA was tested with the qChip Post Oligonucleotide microarray (Quantitative Genomic Medicine Laboratories, Barcelona, Spain). This microarray comprises 60,000 probes mainly located in pericentromeric and subtelomeric regions, as well as regions associated to syndromes caused by recurrent genomic alterations. The average median probes spacing is 35 Kb, and the resolution is of 100–125 Kb. After hybridization with DNA from patient and reference samples, slides were scanned and analyzed for relative gain or loss of fluorescent signals. Genomic region analyses were performed according to the human reference sequence build 37.1 (hg19) with the Genomic Workbench software.

cDNA characterization

RNA of patient 6 was isolated from blood with the PAXgene Blood RNA Kit (Qiagen) following the manufacturer's protocol. The RNA was subsequently reversely transcribed with random primers using the SuperScript® One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. The cDNA was amplified with the forward primer 5′-CCACAAGGTAACTACTCCAG-3 and the reverse primer 5′-CTGCTTCCAGGCACTTTTCTC-3′. The forward primer spans the junction between exon 8 and 9 of *ARID1B*, whereas the reverse primer was designed on the junction between exon 14 and 15. Polymerase chain reaction (PCR) was performed with TaqPolymerase (MP Biomedicals, Santa Ana, CA, USA) with an annealing temperature of 58 °C and 90 s elongation at 72 °C. Gel extraction was performed on the resulting 1331-bp band with the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instruction. The purified fragment was ligated with the shuttle vector pGEM®-T Easy (Promega, Madison, WI, USA) by the use of the T4 DNA Ligase (Promega). *E. coli* DH5α cells were then chemically transformed with the recombinant DNA construct and plated onto LB agar plates containing 100 µg/ml of ampicillin. Transformed bacteria containing the plasmid of interest were grown overnight at 37 °C at 225 rpm in 3 ml of LB medium supplemented with 3 µl of ampicillin. Plasmids were then extracted using the QIAGEN Plasmid Mini Kit (Qiagen) following the manufacturer's instructions. Sanger sequencing was subsequently performed on plasmids extracted from multiple clones.

Results

Mutations in the methyltransferases *KMT2A* and *SETD5* in patients with CdLS-overlapping phenotypes

Patient 1 is a 15-year-old Austrian male with Turkish ancestry (Fig. 1a–c), who received a clinical diagnosis of CdLS during early childhood. At the age of eight, he was evaluated for growth retardation, mild intellectual disability and was noted to have a low anterior temporal hairline, arched eyebrows with synophrys, long eyelashes, ptosis, bulbous nasal tip and thin upper vermillion border of the lip. Minor limb anomalies included small hands, clinodactyly of the fifth finger and syndactyly of toes. The facial features became more pronounced over time, and at the age of 12 years, hairy elbows were also observed. He had an early puberty at the age of 10 years, obstipation and behavioral problems including nocturnal enuresis and encopresis. Targeted gene panel revealed a de novo nonsense mutation in the *KMT2A* gene (NM_001197104) on the blood DNA of the patient. The variant, namely c.8590C > T, p.(Gln2864*), falls within exon 27 and is responsible for the introduction of a premature stop codon. Loss-of-function mutations in the histone methyltransferase KMT2A have been so far described in association with Wiedemann–Steiner Syndrome (WDSTS), in a single patient with CdLS-overlapping features and in a single patient with CSS (Jones et al. 2012; Bramswig et al. 2015; Yuan et al. 2015). In combination with the molecular finding, clinical re-evaluation of our patient at the age of 15 demonstrated features consistent with the diagnosis of WDSTS.

Patient 2 is a 7-year-old German male of Turkish ancestry (Fig. 1d). Clinical features included mild developmental delay, intellectual disability, a low anterior hairline, thick arched eyebrows with synophrys, long eyelashes, a smooth philtrum and thin upper vermillion border of the lip. Taken together, these features suggested a possible clinical diagnosis of a mild form of CdLS. A de novo frameshift deletion in *SETD5* (NM_001080517) was detected on the blood DNA of the patient through whole-exome sequencing. The mutation results in the deletion of two nucleotides in exon 16 (c.2212_2213delAT; p.(Met738Valfs*27)) and is predicted to result in a premature stop codon after 27 amino acids. Clinical re-evaluation of the patient after the molecular finding was still compatible with a mild variant of CdLS.

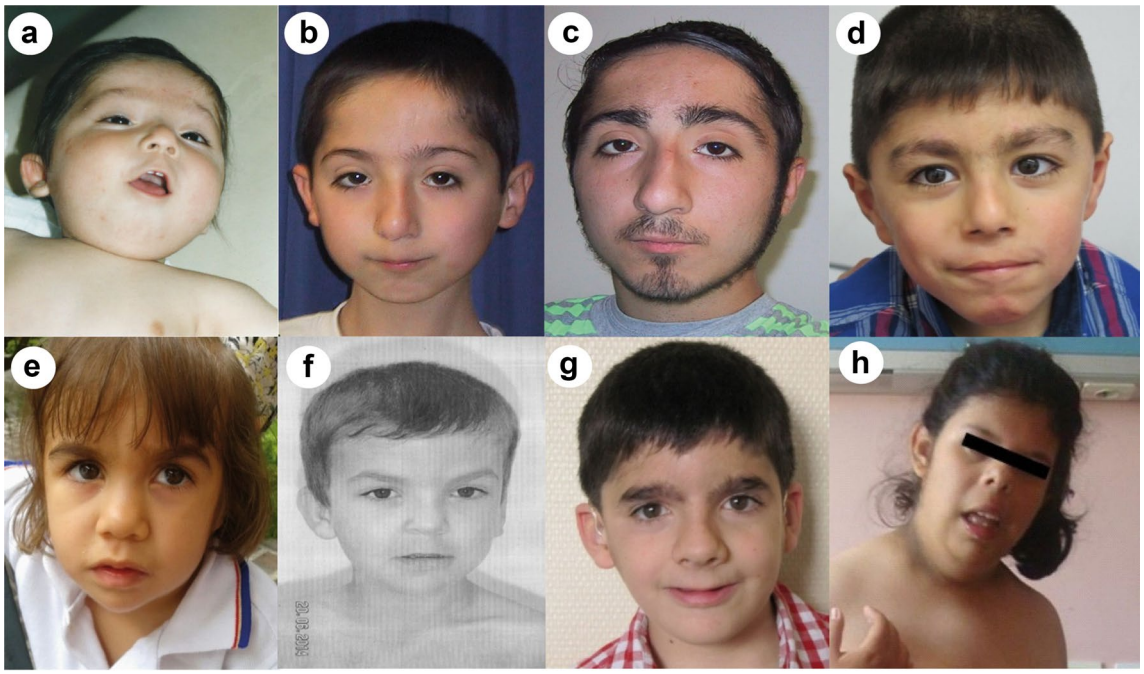

Fig. 1 Phenotype of the patients of our cohort. **a**–**g** Facial appearance of five out of the seven patients with CdLS-overlapping features found to carry mutations in epigenetic genes (writers and remodelers). **h** Facial appearance of the patient clinically diagnosed as Coffin–Siris who turned out to harbor a mutation in *NIPBL*

Consistent with haploinsufficiency of *SETD5* causing CdLS-overlapping features, an intragenic deletion involving *SETD5* was identified in Patient 3 by array-CGH. A 54-kb-spanning deletion (chr3: 9, 457, 143–9, 511, 190, hg19) extends from introns 2 to 19 and results in the loss of the first 16 protein-coding exons. This patient is an 8-year-old German female with Indian ancestry, diagnosed with a CdLS-overlapping phenotype during early childhood. Her clinical features included severely delayed speech and motor development, cognitive impairment, behavioral problems, feeding problems and facial dysmorphisms that included thick eyebrows, long eyelashes, a depressed nasal bridge, broad nasal tip, smooth philtrum and thin upper vermillion border of the lip. The clinical overlap of this patient's features with CdLS was confirmed at a recent evaluation. Loss of the methyltransferase-encoding gene *SETD5* is thought to be central in causing the 3p25 microdeletion syndrome, characterized by intellectual disability, short stature, microcephaly, hypotonia and heart defects (Kuechler et al. 2015); in addition, loss-of-function mutations of *SETD5* have been associated with syndromic and non-specific intellectual disability (Grozeva et al. 2014; Szczaluba et al. 2016). Herein, our data suggest, for the first time, a role of *SETD5* in the etiology of CdLS-overlapping features.

Mutations in both cohesin and SWI/SNF complex members result in overlapping clinical phenotypes

Four patients of our CdLS-overlapping cohort were found to carry mutations in subunits of the SWI/SNF chromatin-remodeler complex, a cellular mechanism linked to the neurodevelopmental disorders CSS and NCBRS.

Patient 4 is a 26-year-old German female who presented with marked pre- and post-natal growth retardation, microcephaly, mild developmental delay, intellectual disability, visual and hearing problems, cardiac and gastrointestinal malformations and minor limb anomalies, including proximally set thumbs, clinodactyly of the fifth finger, syndactyly of toes and hypoplastic nails. Her facial dysmorphisms led to the clinical diagnosis of CdLS. These included a low anterior hairline, thick arched eyebrows, synophrys, depressed nasal bridge, long philtrum and thin vermillion border of the lips. A targeted gene panel revealed the presence of a missense substitution in *SMARCB1* (NM_001317946), namely c.998A > G, p.(Lys333Arg). Similar to the other four missense substitutions in *SMARCB1* previously described in CSS/NCBRS, this mutation falls within the functional SNF5 domain at the C-terminal of the protein (Santen et al. 2012; Tsurusaki

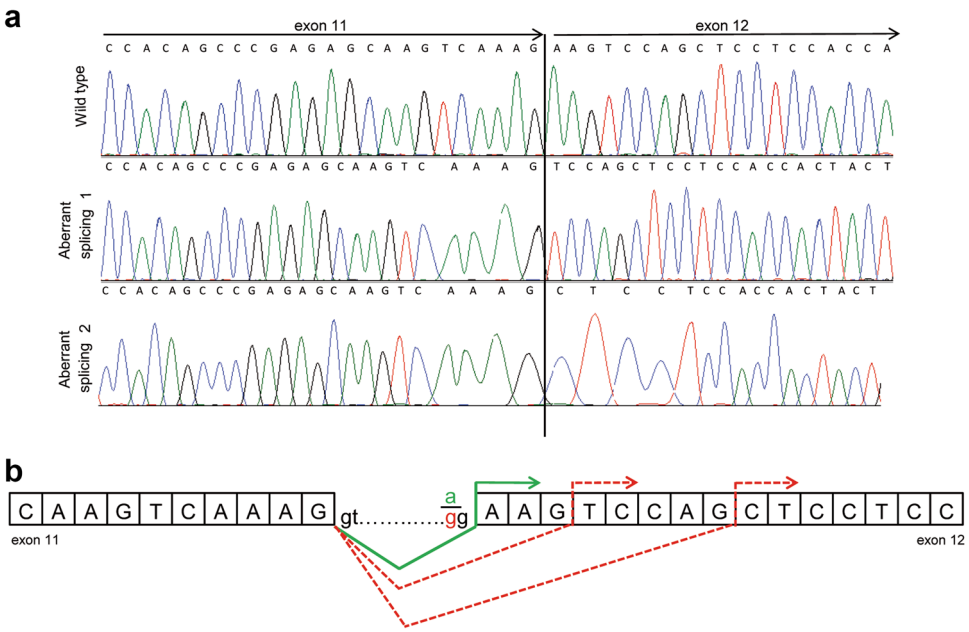

Fig. 2 cDNA analysis for the characterization of the splicing variant of patient 6 (*ARID1B*, c.3136-2A > G). Forward and reverse primers were designed on the junctions between exons 8 and 9 and exons 14 and 15, respectively. The expected wild-type product consisted of 1331 bp. Following amplification, a single 1331-bp band was detected in both control cDNA and patient cDNA. The PCR product of the patient underwent gel extraction and subsequent cloning in the pGEM®-T Easy Vector System. After transformation of DH5α strains of *E. coli*, sequencing analysis of different clones revealed the existence of three distinct splicing isoforms in the patient. **a** Electropherograms of the three different transcripts of patient 6. Besides the wild-type isoform, two aberrantly spliced transcripts were identified, characterized by a different start site in exon 12. **b** Graphical representation of the splicing isoforms of patient 3. *Capital letters* were used to indicate nucleotides of exons 11 and 12, respectively. *Small letters* were used to indicate the donor and the acceptor sites. The wild-type splicing is indicated by the *continuous green line*. The *dotted red lines* indicate, instead, the two aberrantly spliced transcripts. The first transcript is characterized by the deletion of the first three nucleotides of exon 12, thus resulting in an in-frame deletion of one amino acid (p.(Lys1046del)); rather, the second transcript lacks the first eight nucleotides of exon 12, resulting in the out-of-frame deletion p.(Lys1046Leufs*18)

et al. 2012; Wieczorek et al. 2013). In addition, the mutation was indicated as disease-causing by the bioinformatic prediction tool MutationTaster (http://www.mutationtaster.org/). Despite the presence of hypoplastic nails, clinical re-evaluation of the patient after the molecular diagnosis demonstrated again that her features were more consistent with CdLS than CSS.

Patient 5 (Fig. 1e) is a 2-year-old female from Colombia presenting with CdLS-overlapping features that included post-natal growth retardation, intellectual disability, delayed speech development, anomalies of the cardiac and central nervous systems, visual problems, hypertrichosis, small hands, proximally set thumbs, clinodactyly of the fifth finger and multiple craniofacial findings including brachycephaly, low anterior hairline, thick and arched eyebrows with synophrys, long eyelashes, depressed nasal bridge, anteverted nostrils, long philtrum, thin upper vermillion border of the lip and thick and everted lower lip. Exome sequencing identified a de novo nonsense mutation in *ARID1B* (NM_020732), c.2692C > T, p.(Arg898*), previously reported in a patient with a clinical diagnosis of CSS (Wieczorek et al. 2013). Despite a recent clinical re-evaluation that revealed the existence of phenotypical features observed in patients with CdLS and CSS, no clear clinical diagnosis could be assessed for this patient.

Patient 6 is a 7-year-old male of German ancestry (Fig. 1f). In addition to several facial features characteristic for CdLS such as brachycephaly, low anterior hairline, arched eyebrows, broad nasal tip, smooth philtrum and a thin upper lip, the patient presented with short stature, intellectual disability, behavioral problems and developmental delay. Targeted gene panel identified a de novo splicing variant in *ARID1B* (NM_020732), namely c.3136-2A > G. Subsequent analysis of the cDNA of the patient demonstrated two aberrantly spliced transcripts (Fig. 2), one characterized by the deletion of the first three nucleotides of exon 12, resulting in an in-frame deletion of one amino acid (p.(Lys1046del)). The second transcript lacks the first eight nucleotides of exon 12, resulting in an out-of-frame deletion (p.(Lys1046Leufs*18)). Clinical re-evaluation of the patient after the molecular finding confirmed the better fit with a diagnosis of atypical CdLS than CSS.

Patient 7 is a 13-year-old male from Spain (Fig. 1g), who received a clinical diagnosis of CdLS during early

childhood. He presented with pre- and post-natal growth retardation, cognitive impairment, mild developmental delay, behavioral problems, cardiac and genitourinary malformations, limb anomalies (brachydactyly, proximally set thumbs, clinodactyly of the fifth finger and hypoplastic nails), feeding and hearing problems, microcephaly and facial features that included a low anterior hairline, thick eyebrows with synophrys, long eyelashes, anteverted nares, smooth philtrum, thin upper vermillion border of the lip and thick lower lip. By array-CGH, he was found to carry a de novo interstitial deletion on chromosome 6 of 4.1 Mb (chr6: 157, 336, 025–161, 564, 564, hg19) that comprised 34 RefSeq genes including A*RID1B*. A similar deletion containing A*RID1B, ZDHHC14, SNX9, SYNJ2, SERAC1, GTF2H5,* and *TULP4* was previously described in a patient with a clinical diagnosis of CSS (Wieczorek et al. 2013). Interstitial deletions at 6q25 are responsible for a microdeletion syndrome characterized by cognitive impairment, developmental delay, dysmorphic features, structural anomalies of the brain and hearing loss. The critical region for the interstitial 6q microdeletion phenotype was initially mapped to 6q24–6q25 and then further restricted to a 6q25.3 region containing only the protein-coding genes *ARID1B* and *ZDHHC14* (Eash et al. 2005; Nagamani et al. 2009; Michelson et al. 2012). Very recently, haploinsufficiency of *ARID1B* has been recognized as the main mechanism responsible for the phenotypical manifestations of the 6q25 microdeletion syndrome (Ronzoni et al. 2016). Clinical re-evaluation of our patient after the molecular diagnosis indicated a likely diagnosis of CSS.

The clinical overlap observed between CdLS and CSS/NCBRS, along with the identification of mutations in SWI/SNF subunits in patients with CdLS-overlapping features, prompted us to postulate that patients with a likely diagnosis of CSS/NCBRS might accordingly carry mutations in cohesin proteins. To test this hypothesis, eight CSS/NCBRS patients with no previous molecular diagnosis after SWI/SNF and array screening were analyzed by a targeted gene panel that included all known CdLS genes. By this, we identified a missense substitution in *NIPBL* in one German female of Turkish ancestry who was diagnosed as CSS (Patient 8, Fig. 1h) and was previously described (patient K2633 in Wieczorek et al. 2013). The mutation c.6886A > G; p.(Ser2296Gly), that falls in exon 40 of *NIPBL*, alters a region frequently mutated in patients with CdLS (Gillis et al. 2004; Selicorni et al. 2007; Pié et al. 2010) and was predicted as disease-causing by the bioinformatic prediction tool MutationTaster. However, the clinical features of this patient are quite consistent with the initial diagnosis of CSS and include coarse facies with frontal bossing, thick eyebrows, a broad nasal tip with anteverted nostrils, a wide mouth with a thin upper vermillion border of the lip and a thick and everted lower lip and hypoplastic nails.

Discussion

In this work, we demonstrate a remarkable and consistent overlap of molecular and clinical findings of individuals with mutations in cohesin, SWI/SNF complex members and methyltransferases. This observation was developed from the analysis of patients with a tentative diagnosis of CdLS, a neurodevelopmental multisystem disorder characterized by specific facial features and a wide range of overlapping phenotypes (Izumi 2016). Mutations in genes encoding either subunits (*SMC1A*, *SMC3*, *RAD21*) or regulators (*NIPBL*, *HDAC8*) of the cohesin complex have been shown to cause different subentities of the broad CdLS spectrum (Krantz et al. 2004; Tonkin et al. 2004; Musio et al. 2006; Deardorff et al. 2007, 2012a, b). The wide-ranging effects of cohesin mutations are not surprising, as cohesin is a highly conserved protein complex involved in multiple aspects of chromatin biology, including sister chromatid cohesion, transcriptional regulation, long-distance genomic interactions, chromatin-remodeling, DNA damage response and chromosome condensation (Mehta et al. 2013). Mutations in *NIPBL* account for more than half of the patients with CdLS (Gillis et al. 2004; Selicorni et al. 2007; Pié et al. 2010), and these individuals tend to present with a more typical facial appearance and limb deficiencies. These patients can have a phenotype that encompasses a range of mild to severe developmental and cognitive delay, often with absence of speech, and severe pre- and post-natal growth retardation (Selicorni et al. 2007). Mutations in the remaining four genes are found in about 10% of the patients with CdLS and are associated with fewer major structural malformations and less growth retardation, although their intellectual disability can range from mild to profound (Deardorff et al. 2012a, b; Gervasini et al. 2013; Kaiser et al. 2014; Gil-Rodríguez et al. 2015; Parenti et al. 2016b). These latter patients frequently demonstrate clinical features that overlap those of patients carrying mutations in other chromatin-associated proteins. Accordingly, mutations in various chromatin-associated factors aside from cohesin, have been identified in patients with CdLS or CdLS-overlapping phenotypes. These genes include *EP300*, *AFF4*, *ANKRD11* and *KMT2A* (Ansari et al. 2014; Woods et al. 2014; Izumi et al. 2015; Yuan et al. 2015; Parenti et al. 2016a). Notably, mutations in all of these genes have previously been reported in association with other multisystem developmental disorders that are frequently included in the differential diagnosis for CdLS (Roelfsema et al. 2005; Sirmaci et al. 2011; Jones et al. 2012; Izumi et al. 2015). Correspondingly, in our cohort of patients with features resembling CdLS, we identified one patient with a nonsense mutation in the methyltransferase-encoding gene *KMT2A*. Currently, mutations in this gene have been described in association with WDSTS (Jones et al. 2012).

Also, Yuan and colleagues recently identified a mutation in this gene in a patient with features consistent with a diagnosis of CdLS, whereas another study identified a mutation in *KMT2A* in a patient with a tentative diagnosis of CSS (Bramswig et al. 2015; Yuan et al. 2015). Our patient shares with these previously published patients a number of clinical features including short stature, intellectual disability, hypertrichosis, fifth finger clinodactyly, long eyelashes, long philtrum and thin upper lip. However, our patient also demonstrates the presence of hairy elbows, a feature typical for WDSTS that was not observed in the patients described in 2015 (Bramswig et al. 2015; Yuan et al. 2015).

In addition to *KMT2A*, we have also identified loss-of-function mutations in the chromatin regulatory methyltransferase gene *SETD5* in two patients of our cohort. Haploinsufficiency of *SETD5* has been shown to underlie the 3p25 microdeletion syndrome and loss-of-function mutations of this gene have also been shown to cause syndromic and non-specific intellectual disability (Grozeva et al. 2014; Kuechler et al. 2015; Szczaluba et al. 2016). The phenotypic features of our patients with mutations in *SETD5* share similarities with the previously described patients, including a variable level of cognitive impairment and intellectual disability, ear anomalies, abnormal shape of the nose and long philtrum (Grozeva et al. 2014; Kuechler et al. 2015; Szczaluba et al. 2016). Our findings underscore the importance of methyltransferases in chromatin remodeling and suggest that mutations in these genes might alter chromatin regulation in a similar manner as cohesin, resulting in a spectrum of clinical disorders that displays pronounced overlap.

In addition, for the very first time, we have established a direct clinical link to support the recently unveiled molecular link between CdLS and CSS/NCBRS (Lopez-Serra et al. 2014) through the identification of four patients with CdLS-overlapping features with mutations in SWI/SNF subunits and of one CSS patient with a mutation in *NIPBL*. We postulate that additional mutations will be identified with the analysis of a larger number of patients. In our cohort, we have observed overlapping features of CdLS and CSS/NCBRS in each individual and, frequently, several unique features typical of each syndrome. For instance, patients 4 and 7 presented with hypoplastic nails, a feature that is frequently described in association with CSS/NCBRS. In contrast, patients 5 and 6 demonstrated brachycephaly, a finding mainly described in CdLS. These findings indicate that the high degree of clinical overlap of all three syndromes may be explained at least partially by the significant overlap of functions between cohesin and SWI/SNF, both involved in transcriptional regulation, chromatin remodeling and epigenetic modifications. This model is further supported by the direct physical interaction identified between these two protein complexes that has been recently detected in yeast, where the SWI/SNF complex recruits the cohesin loader NIPBL onto broad nucleosome-free chromatin regions that NIPBL subsequently helps to maintain in a nucleosome-free transcriptionally active state (Lopez-Serra et al. 2014).

Notably, three out of the eight patients of our cohort are of Turkish ancestry (patients 1, 2 and 8). Clinical features typical for CdLS such as a synophrys and/or hirsutism might be more frequently observed in individuals of this ethnicity. All of the three patients described here do, indeed, present with synophrys. For this reason, a systematic evaluation of the ethnic background is recommendable for the assessment of the most appropriate clinical diagnosis.

In addition, our findings further support previous observations indicating no clear correlation of the genetic variant with the clinical manifestation of the phenotype, possibly due to the broad variability of clinical features seen in patients with CdLS. Therefore, we strongly recommend the application of next-generation sequencing technologies such as exome sequencing or gene panel sequencing including multiple chromatin regulators to allow the most efficient molecular analysis of patients with a clinical diagnosis of CdLS or CdLS-like phenotypes.

In summary, the identification of mutations in a growing number of chromatin-associated regulators in developmental syndromes strongly indicates that shared functions of these distinct gene products act in common cellular mechanisms and pathways. As a consequence, syndromes caused by mutations in these genes are subsequently characterized by significantly overlapping phenotypes that may be difficult to discern from one another. We propose that these disorders could be aptly grouped into the category of "chromatin dysregulation disorders". The identification of the molecular mechanisms will be of utmost importance for making accurate clinical and molecular diagnoses. Furthermore, understanding the pathogenesis will be essential to develop treatments for individuals with a wide range of clinical features caused by disruption of chromatin regulation.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

References

Ansari M, Poke G, Ferry Q, Williamson K, Aldridge R, Meynert AM, Bengani H, Chan CY, Kayserili H, Avci S, Hennekam RC, Lampe AK, Redeker E, Homfray T, Ross A, FalkenbergSmeland M, Mansour S, Parker MJ, Cook JA, Splitt M, Fisher RB, Fryer A, Magee AC, Wilkie A, Barnicoat A, Brady AF, Cooper NS, Mercer C, Deshpande C, Bennett CP, Pilz DT, Ruddy D, Cilliers D, Johnson DS, Josifova D, Rosser E, Thompson EM, Wakeling E, Kinning E, Stewart F, Flinter F, Girisha KM, Cox H, Firth HV, Kingston H, Wee JS, Hurst JA, Clayton-Smith J, Tolmie J, Vogt J, Tatton-Brown K, Chandler K, Prescott K, Wilson L, Behnam M, McEntagart M, Davidson R, Lynch SA, Sisodiya S, Mehta SG, McKee SA, Mohammed S, Holden S, Park SM, Holder SE, Harrison V, McConnell V, Lam WK, Green AJ, Donnai D, Bitner-Glindzicz M, Donnelly DE, Nellåker C, Taylor MS, FitzPatrick DR (2014) Genetic heterogeneity in Cornelia de Lange syndrome (CdLS) and CdLS-like phenotypes with observed and predicted levels of mosaicism. J Med Genet 51(10):659–668. doi:10.1136/jmedgenet-2014-102573

Bramswig NC, Lüdecke HJ, Alanay Y, Albrecht B, Barthelmie A, Boduroglu K, Braunholz D, Caliebe A, Chrzanowska KH, Czeschik JC, Endele S, Graf E, Guillén-Navarro E, Kiper PÖ, López-González V, Parenti I, Pozojevic J, Utine GE, Wieland T, Kaiser FJ, Wollnik B, Strom TM, Wieczorek D (2015) Exome sequencing unravels unexpected differential diagnoses in individuals with the tentative diagnosis of Coffin–Siris and Nicolaides–Baraitser syndromes. Hum Genet 134(6):553–568. doi:10.1007/s00439-015-1535-8

Braunholz D, Obieglo C, Parenti I, Pozojevic J, Eckhold J, Reiz B, Braenne I, Wendt KS, Watrin E, Vodopiutz J, Rieder H, Gillessen-Kaesbach G, Kaiser FJ (2015) Hidden mutations in Cornelia de Lange syndrome limitations of Sanger sequencing in molecular diagnostics. Hum Mutat 36(1):26–29. doi:10.1002/humu.22685

Castronovo P, Gervasini C, Cereda A, Masciadri M, Milani D, Russo S, Selicorni A, Larizza L (2009) Premature chromatid separation is not a useful diagnostic marker for Cornelia de Lange syndrome. Chromosome Res 17(6):763–771. doi:10.1007/s10577-009-9066-6

Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodríguez C, Arnedo M, Loeys B, Kline AD, Wilson M, Lillquist K, Siu V, Ramos FJ, Musio A, Jackson LS, Dorsett D, Krantz ID (2007) Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of Cornelia de Lange syndrome with predominant mental retardation. Am J Hum Genet 80(3):485–494. doi:10.1086/511888

Deardorff MA, Wilde JJ, Albrecht M, Dickinson E, Tennstedt S, Braunholz D, Mönnich M, Yan Y, Xu W, Gil-Rodríguez MC, Clark D, Hakonarson H, Halbach S, Michelis LD, Rampuria A, Rossier E, Spranger S, Van Maldergem L, Lynch SA, Gillessen-Kaesbach G, Lüdecke HJ, Ramsay RG, McKay MJ, Krantz ID, Xu H, Horsfield JA, Kaiser FJ (2012a) RAD21 mutations cause a human cohesinopathy. Am J Hum Genet 90(6):1014–1027. doi:10.1016/j.ajhg.2012.04.019

Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, Saitoh K, Komata M, Katou Y, Clark D, Cole KE, De Baere E, Decroos C, Di Donato N, Ernst S, Francey LJ, Gyftodimou Y, Hirashima K, Hullings M, Ishikawa Y, Jaulin C, Kaur M, Kiyono T, Lombardi PM, Magnaghi-Jaulin L, Mortier GR, Nozaki N, Petersen MB, Seimiya H, Siu VM, Suzuki Y, Takagaki K, Wilde JJ, Willems PJ, Prigent C, Gillessen-Kaesbach G, Christianson DW, Kaiser FJ, Jackson LG, Hirota T, Krantz ID, Shirahige K (2012b) HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature 489(7415):313–317. doi:10.1038/nature11316

Eash D, Waggoner D, Chung J, Stevenson D, Martin CL (2005) Calibration of 6q subtelomere deletions to define genotype/phenotype correlations. Clin Genet 67(5):396–403. doi:10.1111/j.1399-0004.2005.00424.x

Gervasini C, Russo S, Cereda A, Parenti I, Masciadri M, Azzollini J, Melis D, Aravena T, Doray B, Ferrarini A, Garavelli L, Selicorni A, Larizza L (2013) Cornelia de Lange individuals with new and recurrent SMC1A mutations enhance delineation of mutation repertoire and phenotypic spectrum. Am J Med Genet A 161A(11):2909–2919. doi:10.1002/ajmg.a.36252

Gillis LA, McCallum J, Kaur M, DeScipio C, Yaeger D, Mariani A, Kline AD, Li HH, Devoto M, Jackson LG, Krantz ID (2004) NIPBL mutational analysis in 120 individuals with Cornelia de Lange syndrome and evaluation of genotype-phenotype correlations. Am J Hum Genet 75(4):610–623. doi:10.1086/424698

Gil-Rodríguez MC, Deardorff MA, Ansari M, Tan CA, Parenti I, Baquero-Montoya C, Ousager LB, Puisac B, Hernández-Marcos M, Teresa-Rodrigo ME, Marcos-Alcalde I, Wesselink JJ, Lusa-Bernal S, Bijlsma EK, Braunholz D, Bueno-Martinez I, Clark D, Cooper NS, Curry CJ, Fisher R, Fryer A, Ganesh J, Gervasini C, Gillessen-Kaesbach G, Guo Y, Hakonarson H, Hopkin RJ, Kaur M, Keating BJ, Kibaek M, Kinning E, Kleefstra T, Kline AD, Kuchinskaya E, Larizza L, Li YR, Liu X, Mariani M, Picker JD, Pié Á, Pozojevic J, Queralt E, Richer J, Roeder E, Sinha A, Scott RH, So J, Wusik KA, Wilson L, Zhang J, Gómez-Puertas P, Casale CH, Ström L, Selicorni A, Ramos FJ, Jackson LG, Krantz ID, Das S, Hennekam RC, Kaiser FJ, FitzPatrick DR, Pié J (2015) De novo heterozygous mutations in SMC3 cause a range of Cornelia de Lange syndrome-overlapping phenotypes. Hum Mutat 36(4):454–462. doi:10.1002/humu.22761

Grozeva D, Carss K, Spasic-Boskovic O, Parker MJ, Archer H, Firth HV, Park SM, Canham N, Holder SE, Wilson M, Hackett A, Field M, Floyd JA, UK10K Consortium,Hurles M, Raymond FL (2014) De novo loss-of-function mutations in SETD5, encoding a methyltransferase in a 3p25 microdeletion syndrome critical region, cause intellectual disability. Am J Hum Genet 94(4):618–24. doi:10.1016/j.ajhg.2014.03.006

Izumi K (2016) Disorders of transcriptional regulation: an emerging category of multiple malformation syndromes. Mol Syndromol 7(5):262–273. doi:10.1159/000448747

Izumi K, Nakato R, Zhang Z, Edmondson AC, Noon S, Dulik MC, Rajagopalan R, Venditti CP, Gripp K, Samanich J, Zackai EH, Deardorff MA, Clark D, Allen JL, Dorsett D, Misulovin Z, Komata M, Bando M, Kaur M, Katou Y, Shirahige K, Krantz ID (2015) Germline gain-of-function mutations in AFF4 cause a developmental syndrome functionally linking the super elongation complex and cohesin. Nat Genet 47(4):338–344. doi:10.1038/ng.3229

Jones WD, Dafou D, McEntagart M, Woollard WJ, Elmslie FV, Holder-Espinasse M, Irving M, Saggar AK, Smithson S, Trembath RC, Deshpande C, Simpson MA (2012) De novo mutations in MLL cause Wiedemann–Steiner syndrome. Am J Hum Genet 91(2):358–364. doi:10.1016/j.ajhg.2012.06.008

Kaiser FJ, Ansari M, Braunholz D, Concepción Gil-Rodríguez M, Decroos C, Wilde JJ, Fincher CT, Kaur M, Bando M, Amor

DJ, Atwal PS, Bahlo M, Bowman CM, Bradley JJ, Brunner HG, Clark D, Del Campo M, Di Donato N, Diakumis P, Dubbs H, Dyment DA, Eckhold J, Ernst S, Ferreira JC, Francey LJ, Gehlken U, Guillén-Navarro E, Gyftodimou Y, Hall BD, Hennekam R, Hudgins L, Hullings M, Hunter JM, Yntema H, Innes AM, Kline AD, Krumina Z, Lee H, Leppig K, Lynch SA, Mallozzi MB, Mannini L, McKee S, Mehta SG, Micule I; Care4Rare Canada Consortium., Mohammed S, Moran E, Mortier GR, Moser JA, Noon SE, Nozaki N, Nunes L, Pappas JG, Penney LS, Pérez-Aytés A, Petersen MB, Puisac B, Revencu N, Roeder E, Saitta S, Scheuerle AE, Schindeler KL, Siu VM, Stark Z, Strom SP, Thiese H, Vater I, Willems P, Williamson K, Wilson LC; University of Washington Center for Mendelian Genomics., Hakonarson H, Quintero-Rivera F, Wierzba J, Musio A, Gillessen-Kaesbach G, Ramos FJ, Jackson LG, Shirahige K, Pié J, Christianson DW, Krantz ID, Fitzpatrick DR, Deardorff MA (2014) Loss-of-function HDAC8 mutations cause a phenotypic spectrum of Cornelia de Lange syndrome-like features, ocular hypertelorism, large fontanelle and X-linked inheritance. Hum Mol Genet 23(11):2888–2900. doi: 10.1093/hmg/ddu002

Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, Nowaczyk MJ, Toriello H, Bamshad MJ, Carey JC, Rappaport E, Kawauchi S, Lander AD, Calof AL, Li HH, Devoto M, Jackson LG (2004) Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of *Drosophila* melanogaster Nipped-B. Nat Genet 36(6):631–635. doi:10.1038/ng1364

Kuechler A, Zink AM, Wieland T, Lüdecke HJ, Cremer K, Salviati L, Magini P, Najafi K, Zweier C, Czeschik JC, Aretz S, Endele S, Tamburrino F, Pinato C, Clementi M, Gundlach J, Maylahn C, Mazzanti L, Wohlleber E, Schwarzmayr T, Kariminejad R, Schlessinger A, Wieczorek D, Strom TM, Novarino G, Engels H (2015) Loss-of-function variants of SETD5 cause intellectual disability and the core phenotype of microdeletion 3p25.3 syndrome. Eur J Hum Genet 23(6):753–760. doi:10.1038/ejhg.2014.165

Li H, Durbin R (2010) Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26:589–595. doi:10.1093/bioinformatics/btp698

Liu J, Krantz ID (2009) Cornelia de Lange syndrome, cohesin, and beyond. Clin Genet 76(4):303–314. doi:10.1111/j.1399-0004.2009.01271.x

Lopez-Serra L, Kelly G, Patel H, Stewart A, Uhlmann F (2014) The Scc2–Scc4 complex acts in sister chromatid cohesion and transcriptional regulation by maintaining nucleosome-free regions. Nat Genet 46(10):1147–1151. doi:10.1038/ng.3080

Mehta GD, Kumar R, Srivastava S, Ghosh SK (2013) Cohesin: functions beyond sister chromatid cohesion. FEBS Lett 587(15):2299–2312. doi:10.1016/j.febslet.2013.06.035

Michelson M, Ben-Sasson A, Vinkler C, Leshinsky-Silver E, Netzer I, Frumkin A, Kivity S, Lerman-Sagie T, Lev D (2012) Delineation of the interstitial 6q25 microdeletion syndrome: refinement of the critical causative region. Am J Med Genet A 158A(6):1395–1399. doi:10.1002/ajmg.a.35361

Musio A, Selicorni A, Focarelli ML, Gervasini C, Milani D, Russo S, Vezzoni P, Larizza L (2006) X-linked Cornelia de Lange syndrome owing to SMC1L1 mutations. Nat Genet 38(5):528–530. doi:10.1038/ng1779

Nagamani SC, Erez A, Eng C, Ou Z, Chinault C, Workman L, Coldwell J, Stankiewicz P, Patel A, Lupski JR, Cheung SW (2009) Interstitial deletion of 6q25.2-q25.3: a novel microdeletion syndrome associated with microcephaly, developmental delay, dysmorphic features and hearing loss. Eur J Hum Genet 17(5):573–581. doi:10.1038/ejhg.2008.220

Parenti I, Gervasini C, Pozojevic J, Graul-Neumann L, Azzollini J, Braunholz D, Watrin E, Wendt KS, Cereda A, Cittaro D, Gillessen-Kaesbach G, Lazarevic D, Mariani M, Russo S, Werner R, Krawitz P, Larizza L, Selicorni A, Kaiser FJ (2016a) Broadening of cohesinopathies: exome sequencing identifies mutations in ANKRD11 in two patients with Cornelia de Lange-overlapping phenotype. Clin Genet 89(1):74–81. doi:10.1111/cge.12564

Parenti I, Gervasini C, Pozojevic J, Wendt KS, Watrin E, Azzollini J, Braunholz D, Buiting K, Cereda A, Engels H, Garavelli L, Glazar R, Graffmann B, Larizza L, Lüdecke HJ, Mariani M, Masciadri M, Pié J, Ramos FJ, Russo S, Selicorni A, Stefanova M, Strom TM, Werner R, Wierzba J, Zampino G, Gillessen-Kaesbach G, Wieczorek D, Kaiser FJ (2016b) Expanding the clinical spectrum of the 'HDAC8-phenotype'—implications for molecular diagnostics, counseling and risk prediction. Clin Genet 89(5):564–573. doi:10.1111/cge.12717

Pié J, Gil-Rodríguez MC, Ciero M, López-Viñas E, Ribate MP, Arnedo M, Deardorff MA, Puisac B, Legarreta J, de Karam JC, Rubio E, Bueno I, Baldellou A, Calvo MT, Casals N, Olivares JL, Losada A, Hegardt FG, Krantz ID, Gómez-Puertas P, Ramos FJ (2010) Mutations and variants in the cohesion factor genes NIPBL, SMC1A, and SMC3 in a cohort of 30 unrelated patients with Cornelia de Lange syndrome. Am J Med Genet A 152A(4):924–929. doi:10.1002/ajmg.a.33348

Roelfsema JH, White SJ, Ariyürek Y, Bartholdi D, Niedrist D, Papadia F, Bacino CA, den Dunnen JT, van Ommen GJ, Breuning MH, Hennekam RC, Peters DJ (2005) Genetic heterogeneity in Rubinstein–Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. Am J Hum Genet 76(4):572–580. doi:10.1086/429130

Ronzoni L, Tagliaferri F, Tucci A, Baccarin M, Esposito S, Milani D (2016) Interstitial 6q25 microdeletion syndrome: ARID1B is the key gene. Am J Med Genet A170(5):1257–1261. doi:10.1002/ajmg.a.37553

Santen GW, Aten E, Sun Y, Almomani R, Gilissen C, Nielsen M, Kant SG, Snoeck IN, Peeters EA, Hilhorst-Hofstee Y, Wessels MW, den Hollander NS, Ruivenkamp CA, van Ommen GJ, Breuning MH, den Dunnen JT, van Haeringen A, Kriek M (2012) Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin–Siris syndrome. Nat Genet 44(4):379–380. doi:10.1038/ng.2217

Selicorni A, Russo S, Gervasini C, Castronovo P, Milani D, Cavalleri F, Bentivegna A, Masciadri M, Domi A, Divizia MT, Sforzini C, Tarantino E, Memo L, Scarano G, Larizza L (2007) Clinical score of 62 Italian patients with Cornelia de Lange syndrome and correlations with the presence and type of NIPBL mutation. Clin Genet 72(2):98–108. doi:10.1111/j.1399-0004.2007.00832.x

Sirmaci A, Spiliopoulos M, Brancati F, Powell E, Duman D, Abrams A, Bademci G, Agolini E, Guo S, Konuk B, Kavaz A, Blanton S, Digilio MC, Dallapiccola B, Young J, Zuchner S, Tekin M (2011) Mutations in ANKRD11 cause KBG syndrome, characterized by intellectual disability, skeletal malformations, and macrodontia. Am J Hum Genet 89(2):289–294. doi:10.1016/j.ajhg.2011.06.007

Szczałuba K, Brzezinska M, Kot J, Rydzanicz M, Walczak A, Stawiński P, Werner B, Płoski R (2016) SETD5 loss-of-function mutation as a likely cause of a familial syndromic intellectual disability with variable phenotypic expression. Am J Med Genet A170(9):2322–2327. doi:10.1002/ajmg.a.37832

Tang L, Nogales E, Ciferri C (2010) Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. Prog Biophys Mol Biol 102(2–3):122–128. doi:10.1016/j.pbiomolbio.2010.05.001

Tonkin ET, Wang TJ, Lisgo S, Bamshad MJ, Strachan T (2004) NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. Nat Genet 36(6):636–641. doi:10.1038/ng1363

Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, Kaname T, Naritomi K, Kawame H, Wakui K, Fukushima Y, Homma T, Kato M, Hiraki Y, Yamagata T, Yano S, Mizuno S, Sakazume S, Ishii T, Nagai T, Shiina M, Ogata K, Ohta T, Niikawa N, Miyatake S, Okada I, Mizuguchi T, Doi H, Saitsu H, Miyake N, Matsumoto N (2012) Mutations affecting components of the SWI/SNF complex cause Coffin–Siris syndrome. Nat Genet 44(4):376–378. doi:10.1038/ng.2219

Wieczorek D, Bögershausen N, Beleggia F, Steiner-Haldenstätt S, Pohl E, Li Y, Milz E, Martin M, Thiele H, Altmüller J, Alanay Y, Kayserili H, Klein-Hitpass L, Böhringer S, Wollstein A, Albrecht B, Boduroglu K, Caliebe A, Chrzanowska K, Cogulu O, Cristofoli F, Czeschik JC, Devriendt K, Dotti MT, Elcioglu N, Gener B, Goecke TO, Krajewska-Walasek M, Guillén-Navarro E, Hayek J, Houge G, Kilic E, Simsek-Kiper PÖ, López-González V, Kuechler A, Lyonnet S, Mari F, Marozza A, Mathieu Dramard M, Mikat B, Morin G, Morice-Picard F, Ozkinay F, Rauch A, Renieri A, Tinschert S, Utine GE, Vilain C, Vivarelli R, Zweier C, Nürnberg P, Rahmann S, Vermeesch J, Lüdecke HJ, Zeschnigk M, Wollnik B (2013) A comprehensive molecular study on Coffin–Siris and Nicolaides–Baraitser syndromes identifies a broad molecular and clinical spectrum converging on altered chromatin remodeling. Hum Mol Genet 22(25):5121–5135. doi:10.1093/hmg/ddt366

Woods SA, Robinson HB, Kohler LJ, Agamanolis D, Sterbenz G, Khalifa M (2014) Exome sequencing identifies a novel EP300 frame shift mutation in a patient with features that overlap Cornelia de Lange syndrome. Am J Med GenetA 164A(1):251–258. doi:10.1002/ajmg.a.36237

Yuan B, Pehlivan D, Karaca E, Patel N, Charng WL, Gambin T, Gonzaga-Jauregui C, Sutton VR, Yesil G, Bozdogan ST, Tos T, Koparir A, Koparir E, Beck CR, Gu S, Aslan H, Yuregir OO, Al Rubeaan K, Alnaqeb D, Alshammari MJ, Bayram Y, Atik MM, Aydin H, Geckinli BB, Seven M, Ulucan H, Fenercioglu E, Ozen M, Jhangiani S, Muzny DM, Boerwinkle E, Tuysuz B, Alkuraya FS, Gibbs RA, Lupski JR (2015) Global transcriptional disturbances underlie Cornelia de Lange syndrome and related phenotypes. J Clin Invest 125(2):636–651. doi:10.1172/JCI77435