

Mutations in Mediator Complex Genes *CDK8*, *MED12*, *MED13*, and *MEDL13* Mediate Overlapping Developmental Syndromes

To identify genes that are probably causing a rare genetic disorder, medical geneticists could initially only rely on karyotyping hoping to find a de novo translocation, inversion, or deletion. Such a chromosomal rearrangement was thought to either disrupt a gene or its chromosomal context, i.e., a topologically associated domain [Poot and Haaf, 2015; Lupiáñez et al., 2016]. In this way, translocations and an inversion involving chromosomal band 7q35 produced disruptions of *CNTNAP2* in patients with Gilles de la Tourette syndrome or autism [Verkerk et al., 2003; Bakkaloglu et al., 2008; Poot et al., 2010]. An apparently balanced translocation t(6;7)(q16.2;p15.3) disrupted a topologically associated domain affecting the expression of *TWIST1* in a patient with Saethre Chotzen syndrome [Krebs et al., 1997]. A de novo balanced translocation t(12,17)(q24.1;q21) that interrupted the *MED13L* gene was found in a 7-year-old girl with postnatal microcephaly; developmental delay (DD), in particular delayed motor development and ataxia; mental retardation, with nearly absent speech; a ventricular septal defect, open foramen ovale, and coarctation of the aorta [Muncke et al., 2003]. This complex set of phenotypes suggests that *MED13L* is involved in regulation of development at a very early stage. This inference was supported by findings in a patient with a de novo balanced translocation t(12;19)(q24;q12) that interrupted only the *MED13L* gene [Utami et al., 2014]. This patient showed DD, moderate

intellectual disability, very poor speech, absence seizures, cerebral atrophy, and dysmorphic facial features, including a cleft palate, glossoptosis, retrognathia, hypertelorism, flat philtrum, broad nasal bridge, bulbous nose, strabismus, and hirsutism. In addition, multiple limb contractures, camptodactyly, and foot deformities were seen. Cultured cells from this patient showed decreased mRNA and protein levels of *MED13L* compared to controls, which is in agreement with haploinsufficiency of this gene. While the findings from these 2 translocation carriers suggested a *MED13L* haploinsufficiency syndrome, the associated phenotypes appeared rather varied, and a molecular mechanism for this was not clear. Haploinsufficiency syndromes arise if the gene, of which one copy is lost, encodes a protein that is part of a signaling pathway, a protein complex, or a receptor [Veitia, 2010; Poot et al., 2011; Birchler and Veitia, 2012]. *MED13L* is indeed part of a protein complex, the Mediator complex (MC), which regulates transcription of mRNAs by RNA polymerase II [Conaway et al., 2005; Asadollahi et al., 2017; Harper and Taatjes, 2018].

Karyotyping detects genome rearrangements in approximately 9.5% of the children with DD, whereas genome wide copy number profiling finds a probably pathogenic copy number variation (CNV) in around 19% of the referrals [Hochstenbach et al., 2009]. In a girl with DD, a perimembranous ventricular septal defect, and mild hy-

potonia, a heterozygous 1-Mb de novo triplication involving the *MED13L* gene as well as several nonprotein-coding RNA genes and the *MAP1LC3B2* gene was identified [Asadollahi et al., 2013]. In 2 unrelated patients with cardiac anomalies, an inherited heterozygous duplication or a de novo deletion, both encompassing, among other genes, *MED13L* was found [Adegbola et al., 2015]. While these cases share *MED13L* CNVs as a common denominator, they do not rigorously prove that this gene is responsible for the patient's phenotype. High-resolution arrays allowed detection of losses or duplications of a single gene or even an exon [Boone et al., 2010]. Applying this technology, van Haelst et al. [2015] found a patient with delayed motor and speech development, small eyelids, and mild retrognathia, but no cardiac anomalies, who carried a de novo deletion of exons 6–20 of *MED13L*. A second patient with DD, hypertonia of the extremities, a slightly asymmetric face with short, upslanted palpebral fissures, a bulbous nasal tip and protruding tongue, without cardiac or pulmonary anomalies had a de novo mutation in the splice acceptor site of exon 5 of *MED13L*. This resulted in an in-frame deletion of 15 amino acids in the middle of the *MED13L* protein. In 2 unrelated girls with intellectual delay, facial dysmorphisms and cardiac anomalies, one patient carried a 17-kb deletion encompassing exon 2 of *MED13L* and the other a 115-kb deletion encompassing exons 3 and 4 [Asadollahi et al., 2013]. In a cohort of 41 patients, a 7-year-old girl with delayed psychomotor development and nonsyndromic intellectual disability, a de novo heterozygous truncating mutation in the *MED13L* gene was identified [Hamdan et al., 2014]. Finally, among 106 patients with DD, an 18-year-old man with a de novo heterozygous truncating mutation in *MED13L* was found [Redin et al., 2014]. Reviewing patients with intragenic CNVs of *MED13L*, Abegdola et al. [2015] reported 5 deletions, 3 duplications, and 1 truncating mutation. In contrast, 18 patients with a nucleotide variant in *MED13L* have been reported [Asadollahi et al., 2017]. The preponderance of heterozygous intragenic CNVs points towards a possible pathogenic mechanism of *MED13L* inactivation. Intragenic deletions hint at possible interactions with other proteins as has been suggested for *CNTNAP2* and *TCF4* [Bedeschi et al., 2017; Poot, 2019].

A *MED13L* mutation is suspected in patients with a syndrome consisting of DD, in particular speech impairment, a bulbous nasal tip, macroglossia, macrostomia, or an open mouth [Tørring et al., 2019]. Carriers of a *MED13L* mutation affecting CNV harbor the so-called *MED13L* haploinsufficiency syndrome, which in all cases

consists of moderate to severe intellectual delay and facial dysmorphic traits [Assodollahi et al., 2017]. In the majority of these patients, severe speech delay and muscle hypotonia are also noted [Assodollahi et al., 2017]. In roughly 20–50% of the patients, further features include abnormal MRI findings of myelination, an abnormal corpus callosum, ataxia and coordination problems, autism spectrum disorder, seizures and/or abnormal EEG, and variable congenital heart defects. In most patients, facial anomalies include a broad forehead, low-set ears, bitemporal narrowing, upslanting palpebral fissures, depressed or flat nasal bridge, a bulbous nose, and an abnormal chin. In 30% of the cases, macroglossia and horizontal eyebrows were observed. These features are key in distinguishing *MED13L* haploinsufficiency syndrome from the 1p36 deletion and Kleefstra syndromes. The facial gestalt of *MED13L* haploinsufficiency syndrome also bears resemblance with 22q11.2 deletion syndrome (also known as DiGeorge or velocardiofacial syndrome) [Assodollahi et al., 2013; Cafiero et al., 2015].

Since *MED13L*, together with *CDK8*, cyclin C, *MED12*, *MED12L* and *MED13*, forms the regulatory subunit of MC, it is tempting to suggest that either perturbation of interactions of *MED13L* with these proteins or mutations in them will result in comparable syndromes. Patients with protein-altering mutations in the *MED13* gene showed intellectual disability and/or DD, including speech delay or speech-related disorders [Snijders-Blok et al., 2018]. In a cohort of 13 patients, 2 or more showed autism spectrum disorder, attention deficit hyperactivity disorder, optic nerve abnormalities, Duane anomaly, hypotonia, mild congenital heart abnormalities, and dysmorphisms [Snijders-Blok et al., 2018]. In 12 unrelated patients presenting with hypotonia, mild to moderate intellectual disability, behavioral disorders, and variable facial dysmorphism, *CDK8* mutations were found [Calpena et al., 2019]. In several patients, congenital heart disease, agenesis of the corpus callosum, anorectal malformations, seizures, and hearing or visual impairments were detected. In a cohort of 7 patients with intellectual disability and/or DD, including speech impairment and features such as autism spectrum disorder, aggressive behavior, corpus callosum abnormality, and facial dysmorphologies, mutations affecting the *MED12L* gene were found [Nizon et al., 2019]. CNVs were detected in 3 patients, while 4 others harbored single nucleotide variants. Based on their phenotypic presentation, patients with *MED13*, *MED13L*, *MED12L*, or *CDK8* mutations are difficult to distinguish from the *MED12*-related Opitz-Kaveggia, Lujan-Fryns, and Ohdo syndrome [Adegbola et al., 2015;

Caro Llopis et al., 2016; Calpena et al., 2019; Srivastava et al., 2019]. Rather, these syndromes appear to form a continuum, which has been subsumed under the term of “transcriptomopathies” [Yuan et al., 2015; Caro Llopis et al., 2016].

It appears that patients with mutations affecting any proteins forming the regulatory component of MC present with a syndrome with variable expressivity, in particular with respect to congenital heart defects. Functional studies may help to elucidate the effects of genetic alterations of MC. Analysis of cells from 2 individuals with *MED12L* mutations revealed a modest yet significant alteration of *MED12L* mRNA synthesis rates, akin to findings with an intragenic deletion in the *CNTNAP2* gene [Lee et al., 2015; Nizon et al., 2019]. In cells with missense mutations in *CDK8*, *STAT1* phosphorylation is reduced, in most cases to a similar extent as in a kinase-dead control [Calpena et al., 2019]. *MED12* mutations in patients with Opitz-Kaveggia or Lujan syndromes disrupt the *GLI3*-dependent sonic hedgehog (*SHH*) signaling pathway under the control of MC [Zhou et al., 2012]. In lymphoblast cell lines from these patients, expression levels of multiple *SHH/GLI3* target genes such as *ASCL1*, *BMP4*, *CREB5*, and *NEUROG2* were significantly elevated [Zhou et al., 2012]. In patients with X chromosome-linked intellectual delay, different *MED12* mutations caused distinct patterns of altered expression of the *SHH/GLI3* target genes *CREB5*, *BMP4*, and *NEUROG2* [Srivastava et al.,

2019]. *GLI3* is a zinc finger transcription factor with a dual function as transcriptional activator and repressor of the *SHH* system, which is known as a regulator of cranial suture development [Tanimoto et al., 2012; Poot, 2019]. The studies discussed above implicate the regulatory components of MC as a core regulatory mechanism in much more complex developmental syndromes. Assembly of MC cannot only be disrupted by mutations in its component genes, but in mouse liver, it is also sensitive to the nutrition state [Youn et al., 2019]. Thus, during fasting, genetically insulin-resistant and obese mice displayed loss of the kinase module of MC [Youn et al., 2019]. It is not known to what extent this may lead in pregnant women to offspring with DD and other phenotypes of the *MED12*-*MED13L*-*CDK8* continuum. Given the phenotypic overlap between chromosome 1p36 deletion syndrome and 22q11 deletion syndrome with the *MED12*-*MED13L*-*CDK8* syndrome continuum, it may also be worthwhile to study the expression levels of the genes in these deletions in patients with *MED12*, *MED13L*, or *CDK8* mutations. Following a path from karyotyping, via genome wide aneuploidy screening and exome sequencing, we saw individual patients becoming manifestations of syndromes, which merged in an MC-based continuum at the center of a complex and only partly understood regulatory mechanism of development.

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