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Genotype-Phenotype Correlations in Cornelia de Lange Syndrome

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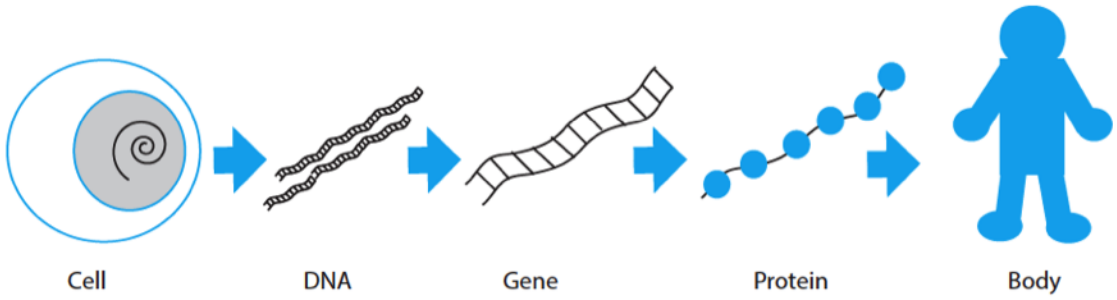
Genetics 101

As a brief background on genetics, our bodies are made up of billions of cells and within each cell there are chromosomes which are the structures that hold all of our ~20,000 genes and genetic information. Genes function as the body's instruction manual telling our body how to grow and develop. We have two copies of each gene as we get one set from our mothers and one set from our fathers. Genes are made up of genetic material, called DNA, and they serve as the blueprint from which proteins are made. Proteins are the basic building blocks of the human body performing specific functions so that our bodies work properly controlling everything from our heartbeat to determining our eye color (Figure 1).



Sometimes changes (or mutations) spontaneously occur in genes that prevent them from working properly altering the proteins that are made. As a result, this affects various bodily functions including growth and development. Currently, mutations in five different genes, *NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21*, have been associated with typical Cornelia de Lange Syndrome (CdLS). Since the discovery of these initial 5 genes, several other genes, such as *AFF4*, *BRD4*, *ANKRD11*, *TAF1* and *TAF6* have been associated with clinical pictures similar to CdLS when mutated.

The association between the presence of a certain mutation or mutations in a specific gene (genotype) and the resulting presence, absence or severity of symptoms or clinical features (phenotype) is called a "genotype- phenotype correlation". As we have known

about the five main genes involved in typical or classic CdLS for some time, certain genotype-phenotype correlations have been observed for each of these genes. With such little information known about the newer CdLS or CdlS-like genes (*AFF4, BRD4, ANKRD11, TAF1* and *TAF6)* genotype-phenotype correlations are more difficult to define.

Overall, changes in each of the genes are found in approximately 84 percent of individuals with a clinical diagnosis or suspicion of CdLS or a CdLS-like diagnosis, with the vast majority being caused by mutations in *NIPBL* [Kline et al. 2018]. Mutations in the *NIPBL, SMC3, RAD21, BRD4, ANKRD11* and *AFF4* genes are inherited in an autosomal dominant pattern meaning one genetic change in only one copy of the gene is enough to cause symptoms. These changes are typically not inherited (or passed down) from a parent and are usually new changes (*de novo*) that occur in a child for the first time spontaneously. *SMC1A, HDAC8,* and TAF*1* are inherited in an X-linked dominant pattern as they are located on the X chromosome. Women have two copies of the X chromosome and men have one copy each of the X and Y chromosome. Since women have two X chromosomes, the risks for future pregnancies are determined by the carrier status of the mother—although in almost all cases of CdLS caused by an X-linked gene, they have been new (or *de novo*) changes and are not carried in the mother. For both the dominant and X-linked genes, if parents are clinically unaffected and do not carry the same genetic mutation as their child the recurrence risk for future pregnancies is ~1%. This is due to "germline mosaicism." In germline mosaicism, the mutation in the gene that causes CdLS arose in a precursor cell that went on to form a group of eggs or sperm. In these rare cases, the change then exists in multiple sperm or eggs but not in other tissues of the parents, so their blood testing will be negative and they will not have features of CdLS. However individuals who are germline mosaic are at risk (as high as 50 percent) to have other children with CdLS. Lastly, *TAF6* is inherited in an autosomal recessive pattern meaning both copies of the *TAF6* gene need to have a genetic change in order to cause clinical symptoms. For recessive genes, the parents are typically unaffected carriers each carrying one of the *TAF6* mutations. Recurrence risk for future pregnancies in this scenario would be 25%.

Somatic Mosaicism

A study led by Dr. Raoul Hennekam in the Netherlands [Huisman et al 2013] has found that mosaicism for *NIPBL* mutations may be found in up to 30 percent of individuals with CdLS who have tested negative in the blood for mutations in the known CdLS genes. Mosaicism means that an individual has a change in a gene which is present in only some but not all of the cells in their body. If an individual is mosaic for a change in *NIPBL*, we may not be able to identify this change by testing only their blood; instead, we may need to test other cells from other tissues such as cheek cells, also called buccal cells.

More recently, somatic mosaicism has also been reported in two other CdLS genes including *SMC1A* and *SMC3* (Ansari et al. 2014). Parenti et al. also reports a case of somatic mosaicism of an *HDAC8* mutation in a mother who had two affected children. There have also been reports of individuals with mosaic mutations in genes responsible

for atypical forms of CdLS with overlapping phenotypes. A young girl with features reminiscent of CdLS, including microcephaly, short stature and characteristic facial features, was found to have a mutation in *ANKRD11* present in a mosaic state (Parenti et al. 2016). Mosaic mutations are most often not detected through standard screening approaches using DNA isolated from blood. Given these findings, complete testing would require examining a different tissue type in addition to blood (when a causative mutation in an individual suspected of having CdLS is not found in the blood) to rule out a mosaic mutation in other tissues.

Depending on the number of cells carrying the mutation and the tissues involved, an individual with mosaicism can theoretically present with a more mild form of CdLS. However, additional research is needed in this area since only a few patients with mosaicism in the CdLS genes have been identified thus far.

NIPBL

Individuals with classic findings of CdLS, including characteristic facial features and limb anomalies, are likely to have a change identified in the *NIPBL* gene. However, changes (or mutations) in *NIPBL* have been found in individuals with both classic and mild presentations. The degree of severity depends on the specific type of mutation that occurs and where the mutation falls within the *NIPBL* gene.

A truncating (or frameshift) mutation is one type of mutation that tends to have a more significant effect on the gene that can ultimately block protein production. Therefore, individuals with truncating mutations typically present with a more classic or severe form of CdLS.

Missense mutations are a different type of mutation which generally only slightly changes the protein. Therefore, individuals with missense mutations typically present with milder forms of CdLS, since their proteins likely retain some residual function.

SMC1A* and *SMC3

Individuals with *SMC1A* or *SMC3* mutations typically have fewer structural differences, such as a limb difference or heart difference. Such individuals also tend to present with less significant growth restriction than those with *NIPBL* mutations. However, individuals with *SMC1A* or *SMC3* mutations will still typically have intellectual disability that can range from moderate to severe [Deardorff et al 2007].

Subtle facial features in individuals with *SMC1A* or *SMC3* mutations may differ than those observed in “classic” CdLS caused by *NIPBL* mutations and can include slightly flatter and broader eyebrows with a broader and longer nasal bridge [Rohatgi et al 2010]. Specifically, individuals with *SMC3* mutations often have subtle or absent synophyrs (connecting eyebrows), wider nose with a rounder tip, and a well-formed philtrum (vertical groove between the base of the nose and upper lip).

As broad-scale genetic testing has increased and many indiviudals with unexplained clinical findings are undergoing genetic testing, including exome sequencing (which sequences all 20, 000 genes in an individual to find answers), we have found some individuals to have changes or mutations in the *SMC1A* gene who do not have CdLS but rather have a severe seizure disorder with intellectual disability [Symonds et al., 2017]. The type of mutation affecteing the SMC1A gene is what causes these different diagnosies. Individuals with CdLS caused by *SMC1A* mutations tend to have missense mutations that likely affect the function of the SMC1A protein whereas individuals with the severe seizures and intellectual disability tend to have mutations that likely knock out the function of the SMC1A protein completely. Since SMC1A is on the X chromosome (and boys have only one X chromosome and girls have two X chromosomes) we generally do not see boys with the severe seizure and intellectual disability clinical picture since they would have no functional SMC1A protein which would generally not be compatible with survival through embryonic development. We do see both males and females with SMC1A-related CdLS since the mutations in SMC1A that cause the CdLS diagnosis tend to result in an SMC1A protein being made but with decreased function than the non-mutated form.

RAD21

Individuals with mutations in *RAD21* typically do not have major structural differences. Individuals with *RAD21* mutations have milder cognitive impairment compared to those with "classic" CdLS. These individuals typically display growth retardation, minor skeletal anomalies, and facial features that overlap with CdLS. [Deardorff et al 2012].

HDAC8

Individuals with mutations in *HDAC8* have facial features which overlap with CdLS but typically display delayed closure of the anterior fontanel (the opening or "soft spot" on the top of the head in babies which typically closes around one year of age), hooded eyelids, a wider nose, varying pattern of skin pigmentation, and friendly personalities. Growth restriction also tends to be less significantly affected with this gene and a lower frequency of microcephaly (small head circumference) is reported.

In females, the severity of clinical presentation caused by mutations in *HDAC8* is variable, since this gene is on the X chromosome and females have two X chromosomes while males have only one X chromosome and a Y chromosome. Since women have two X chromosomes in every cell, they randomly shut off one copy of the X chromosome (called X-inactivation). Therefore, depending on how many X chromosomes with the mutation versus those without the mutation are inactivated will directly influence the severity of their clinical presentation. (Though *SMC1A* is also located on the X chromosome this X-inactivation process does not apply to the *SMC1A* gene).

BRD4

The BRD4 protein is known to interact with the NIPBL protein and mutations in the *BRD4* gene have been recently reported in a few individuals with an atypical CdLS presentation [Kline et al. 2018]. The spectrum of clinical features associated with *BRD4* is unclear with such few cases reported in the literature at this time [Olley et al. 2018]. However, from those that have been found to carry a *BRD4* mutation, significant overlap with CdLS was noted. The key overlapping features observed in those with *BRD4* mutations include intrauterine growth retardation (IUGR), global developmental delay, congenital heart defects (PDA, VSD, ASD), hearing loss, seizures, and gastroesophageal reflux. Overlapping facial features include synophrys, arched eyebrows, short nose and anteverted nostrils. Several findings atypical of classic CdLS reported in these individuals include normal height, preauricular ear tag, supernumerary nipple, hypothyroidism, hyperlipidemia, and a thin corpus callosum.

ANKRD11

KBG syndrome is a neurodevelopmental disorder caused by mutations in the *ANKRD11* gene. Some individuals with mutations in *ANKRD11* have been reported to present with clinical features suggestive of Coffin-Siris syndrome or Cornelia de Lange syndrome. KBG syndrome is characterized by intellectual disability and/or developmental delays, characteristic facial features (triangular face, brachycephaly or a flatter appearing head, synophrys and hypertelorism or widely spaced eyes), large upper central incisors, skeletal anomalies, postnatal short stature, conductive hearing loss, and behavioral abnormalities (such as autism spectrum disorder or hyperactivity) [Swols et al. 2017]. Hearing loss is described in almost 1/3 of individuals with KBG and it can be conductive (CHL) or sensorineural hearing loss (SNHL). Some children can also have costovertebral anomalies (area where the ribs connect to the spinal column), scoliosis and EEG abnormalities with or without seizures. Behavioral difficulties such as hyperactivity, autistic features, aggressive compulsive behavior, and anxiety are also frequently observed. KBG can be quite variable in regard to cognitive abilities of affected individuals, and there have been no reported cases of regression in cognitive abilities. Like CdLS, most children will require support in the classroom, and special education. There have been over 100 cases of KBG syndrome reported, and while it is thought to be quite rare, it is likely undiagnosed due to the mild features present in some affected individuals. Studies suggest that males typically have more severe clinical presentations than females.

TAF1

TAF1 variants have been associated with an X-linked intellectual disability syndrome. Individuals with mutations in *TAF1* present with global developmental delay, generalized hypotonia, and variable neurologic manifestations. Specific facial features associated with this diagnosis include long face, prominent supraorbital ridges (bony ridge above the eye socket), long philtrum (middle area of the upper lip), anteverted nostrils, pointed chin, and protruding ears [O'Rawe et al 2015]. Additional features commonly shared by the individuals reported by O'Rawe et al [2015] include microcephaly (small head circumference) and hearing loss. Some of the features overlap with atypical presentations

of CdLS though with such few individuals reported the full spectrum of characteristics associated with *TAF1* mutations is yet to be identified.

TAF6

Variants in *TAF6* have been associated with an autosomal recessive CdLS-like presentation. Thus far, *TAF6* mutations have been reported in two unrelated families with features reminiscent of CdLS and includes intellectual disability, developmental delay, and short stature. Characteristic facial features overlapping with CdLS include synophrys, arched eyebrows, long eyelashes, and thin upper lip. Again, there is limited clinical information known with only two families reported in the literature at this time.

AFF4

CHOPS syndrome is characterized by *c*ognitive impairment and *c*oarse facies, *h*eart defects, *o*besity, *p*ulmonary involvement, *s*hort stature, and *s*keletal dysplasia [Izumi et al., 2015]. CHOPS syndrome is caused by mutations in the *AFF4* gene. Individuals with CHOPS syndrome have a distinct presentation that is characterized by short stature, characteristic facial features (synophrys, arched eyebrows, long eyelashes, upturned nasal tip with anteverted nostrils and coarse, full facies), congenital heart defects, pulmonary involvement, brachydactyly (short fingers and toes) and other skeletal involvement, genitourinary issues, and developmental delay/intellectual disability. Congenital heart disease, pulmonary, and skeletal issues are the most common complications [Mehta et al, unpublished data]. Obesity, pulmonary involvement, skeletal findings and distinct craniofacial features are the most notable features distinguishing CHOPS syndrome from CdLS. Though all individuals have short stature (which is observed in CdLS), all have high BMIs and many were noted to exhibit food-seeking behaviors. Pulmonary issues are not typically observed in CdLS and the pulmonary involvement for children with CHOPS syndrome can be significant with chronic lung disease present in the majority of individuals with some requiring tracheostomy. Skeletal involvement is the third distinguishing feature with brachydactyly commonly observed. Distinct craniofacial features observed in CHOPS syndrome includes coarse facial features with a round face or a fullness to the face which is not observed in CdLS. While individuals with CHOPS syndrome present with 4 major distinguishing features, they do still have some overlap with CdLS as many of the syndrome's other features such as microcephaly, developmental delay, intellectual disability, heart defects and hearing loss are also frequently observed in those with CdLS. The facial features, especially in younger individuals do have similarities to CdLS including synophrys, long eyelashes, upturned nose and arched eyebrows, as the children get older the coarseness and fullness of the face gives a more characteristic CHOPS gestalt.

What it all means

While clinicians and researchers have reported these genotype-phenotype correlations (as described above) it is important to note that they are generalities that are often true but these "rules" are also frequently broken. We have seen children with severe (truncating or

frameshift) mutations in *NIPBL* who have a mild clinical picture (phenotype) and conversely also seen children with mild (missense) mutations in *NIPBL* who have a very severe clinical picture.

There is still much work to be done to fully understand the correlations between the genotype and phenotype in individuals with CdLS and CdLS-like diagnoses, but there are general principles emerging and clinicians are getting better at using this information to help provide some guidance and prognostic information to families.

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