

The Discovery

Identification of the Cornelia De Lange Syndrome Gene

by Ian D. Krantz, M.D.

After many years of hard and often-times frustrating work we finally have had a breakthrough in our search for the underlying cause of Cornelia de Lange Syndrome (CdLS). In close collaboration with Dr. Laird Jackson, my lab at The Children's Hospital of Philadelphia, identified changes in a gene, that we have named *NIPBL*, in individuals with CdLS. The details of this work have been published in the journal *Nature Genetics* in the June issue under the title "Cornelia de Lange Syndrome is caused by Mutations in *NIPBL*, the human homolog of the *Drosophila Nipped-B* gene."

Several years ago Dr. Jackson and I realized that the region on chromosome 3 that many investigators felt contained the CdLS gene was not clearly associated with CdLS in a number of the families that we were studying. We decided at that time that we should look at the whole genome (all of the chromosomes) for a region associated with CdLS and not restrict our studies to just a small portion of chromosome 3. We undertook a large scale study, called a genome-wide linkage analysis, to look for regions of the genome that were consistently inherited in children with CdLS and not in their unaffected siblings. Dr. Jackson was instrumental in collecting blood samples to carry out these studies from the rare families with more than one family member with CdLS.

After many years of work we were able to identify four regions of the genome that were candidates for containing the CdLS gene (small regions on chromosomes 2, 5, 10 and 14). There were hundreds of genes contained in these regions so in order to see if one of these four candidate regions was more likely to contain the gene we were looking for than the others, we looked for other clues that might point to one region in particular. These clues came in the form of two unrelated children who had chromosomal rearrangements that involved the exact region on chromosome 5 that we found associated in the families we had studied.

Through various studies we were able to narrow down the region on chromosome 5 to a small region that contained 11 genes. At this point we looked at the sequence of all of these genes in children with CdLS and found changes in a large gene that we named *NIPBL*.

While our studies on this new gene are very preliminary at this time there are some important findings that we have already discovered. As had been suspected from studying the inheritance of CdLS over many years, we have shown that having a change in one copy of your *NIPBL* gene is sufficient to cause CdLS (we have two copies of every gene in all of our cells; one from mom and one from dad), even though the other copy of *NIPBL* is fine. We have found changes in this gene in individuals who are both mildly and severely affected with CdLS. At present we have found changes in about half of all children with CdLS that we have tested. For all individuals in whom we have found a change in *NIPBL*, it is always a new change (i.e. not carried by mom or dad) that occurred. In those rare families where there is more than one child with CdLS and neither parent has any features of CdLS, we have found that this is due to something called "germ line mosaicism." This is the situation where the change in *NIPBL* likely occurred in a precursor cell that went on to form a subgroup of sperm or eggs in the parent resulting in a group of eggs or sperm that subsequently had the *NIPBL* change in it. Since the change in *NIPBL* is only in a subset of cells it did not cause CdLS in that parent but resulted in the possibility of passing it on to more than one child. While these types of recurrences in families are very rare (~1% of all families who have a child with CdLS), germ-line mosaicism has been found to be the cause in all of the families with more than one affected child that we have studied. In some instances where a mildly affected parent with CdLS has a child with CdLS we know that the parent has one copy of *NIPBL* changed in all of their cells, and one normal functioning copy. That parent is then at a 50% risk of passing it on to their children (only one copy of the *NIPBL* gene is passed on to any sperm or egg). It is important to stress that the vast majority of families with a child with CdLS do

not have a risk for future affected children, and the change in the *NIPBL* gene in that child is presumed to have arisen as the result of new change in the single egg or sperm that went on to form that child.

Current Research

We are currently performing more studies to see if particular changes in the gene are associated with a more severe or a more mild form of CdLS, so as to have a prognostic indicator for families. We are also developing improved testing methods to see if we can identify changes in this gene in all individuals with CdLS. It is unclear at this time if those individuals with CdLS in whom we are not able to find changes in *NIPBL* actually have a change in this gene that we are missing. Screening for changes in this gene is very difficult as it is a very large gene. We use the analogy of reading a large book and trying to find a single spelling error, it may be there but you may miss it, and for gene testing the ability to find the majority of changes depends on different testing methods. At this time only one technique is being used. There is also the possibility that there may be a second, as yet unidentified, gene that could also cause CdLS when changed.

Future Research

My lab at The Children's Hospital of Philadelphia is working to develop new testing modalities for *NIPBL* as well as to look at the possibility of a second gene existing that may also cause CdLS. Our goal is to be able to identify the underlying molecular change in all individuals with CdLS. We are also actively trying to understand what the *NIPBL* gene does. At this time, based on studies done in the fruit fly, we know that this gene is important in regulating (turning on and off) many other genes. One question that we would like to answer is which genes does *NIPBL* control? We also know that the *NIPBL* gene product (called the *NIPBL* protein) has to interact with other proteins to function normally. We are trying to find out exactly which other proteins interact with *NIPBL*. Answering these questions will allow us to understand exactly how *NIPBL* acts and why changes in the gene result in the clinical manifestations we see in individuals with CdLS. We hope that through understanding its action eventually better treatments and therapies will be developed, however this will likely take quite a bit of time.

One of the immediate advantages of having identified the CdLS gene is that we now have a means to directly test individuals with CdLS by means of a blood test to confirm a diagnosis. If a change is found, then that information can be used by families to screen other family members or, if desired, as a prenatal test for couples wishing to have more children who are concerned about recurrence risks.

The road to this point has been long but very rewarding. Working closely with the Foundation, and experiencing firsthand the generosity and dedication of all the families who have helped advance the research that has led to the identification of the underlying cause has been a truly inspiring experience. The research has been driven by all of you and has been supported by donations directed to research through the Foundation as well as by a large grant from the National Institutes of Health (NIH) through the National Institute of Child Health and Development (NICHD). Funding in the past has been difficult to obtain, but hopefully through this work more funds will be made available to study the *NIPBL* gene and CdLS and many more investigators from around the world will become involved in this research.