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Homing In On a Cause of Blue Baby Syndrome

The most common form of blue baby syndrome, the serious congenital heart defect known as tetralogy of Fallot (TOF), has always been something of a puzzle for biomedical science. Seventy percent of the time, the condition, which causes a newborn's skin to turn blue from lack of oxygen in the blood, arises without any explanation.

Now, a team of researchers led by Howard Hughes Medical Institute investigator Christine Seidman of Brigham and Women's Hospital and Harvard University Medical School, has found genetic clues that narrow the search for the underlying cause of TOF. Many of the genetic culprits, they find, arise in patients without being passed on by the parents. The team's results are reported online July 13, 2009, in the journal *Nature Genetics*.

"The study represents an important step forward in understanding the cause of tetralogy of Fallot," says Seidman, who led the study by an international group of researchers with her husband, former HHMI investigator Jonathan Seidman, also of Harvard Medical School. "Until now, the etiologies of TOF have been largely unknown."

"Copy number variations may help us to identify genes that cause other serious birth defects."

- Christine E. Seidman

Babies affected by TOF have malformed hearts that cannot fully oxygenate the blood. This causes cyanosis, a blue coloration of the skin due to deoxygenated hemoglobin in blood vessels near the skin surface. TOF can be treated surgically, but remains a serious, lifelong problem and frequently results in early mortality. It occurs in about one in 3,000 live births, and accounts for 10 percent of all serious congenital heart disease.

TOF is puzzling, Seidman says, because the parents of most babies with the condition have normal hearts and seem to confer no genetic predisposition for

the defect. Some instances of TOF have been blamed on prenatal infection, maternal illness, or exposure to chemicals that interfere with an embryo's development, but the majority of cases are unexplained.

Some cases of the condition have been linked to mutations in known heart-related genes, though those particular mutations do not consistently cause TOF. These hints at a genetic component, however, spurred Seidman and colleagues to scour the genomes of 114 TOF patients in search of more definitive answers. The group identified 11 segments of DNA that, in some patients, were present in too many or too few copies – not the standard two copies found in individuals without TOF.

These kinds of genetic changes, known as copy number variations, alter the "dose" of a given gene -- changing how much of its protein is produced by a cell. Gains or losses of large chunks of DNA have been linked a number of disorders, and specific copy number variations are known to increase the risk of autism, schizophrenia, and certain cancers, and alter individuals' susceptibility to HIV. But they can be hard to find.

"Until very recently, to a large extent, we didn't know these things existed," Seidman says. However, newly available technology for detecting variations in genetic sequence – a specialized gene chip -- has given researchers the ability to scan whole genomes and look for copy number variations.

The next step for Seidman's team was to determine whether these copy number variations were present in the genomes of other patients with TOF. They examined the DNA of another 398 patients, and concluded that seven of the copy number variations they had identified substantially increased the risk of TOF. One example is a gene called RAF1, which influences the growth, movement, and survival of cells. Excess copies of RAF increase the risk of TOF nine-fold. "Just changing the dose of that one gene causes tetralogy of Fallot," Seidman says.

Many of the copy number variations that the team detected were large, spanning multiple genes. Those regions of DNA, they say, should be prioritized for future study so that researchers can zero in on exactly which of their genes contribute to TOF.

Notably, Seidman's team also analyzed the DNA of patients' parents – none of whom had TOF. The copy number variations they found in the patients' DNA were, in almost all cases, not present in the parents' DNA. As many as ten percent of the patients in their analysis had copy number variations that were not inherited from their parents. That, Seidman says, helps explain a diverse genetic underpinning for the serious heart malformation.

According to Seidman, the study shows that new molecular techniques can now be used to comb genomes in exquisite detail and identify very small

differences in DNA that can have big effects. The strategy, she says, can be applied to other conditions and help identify the molecular underpinning of diseases whose causes are unknown. "Copy number variations can provide clues for studying other congenital malformations," says

Seidman. "That's the other take home message of the study. Copy number variations may help us to identify genes that cause other serious birth defects."