Many children with kidney disease have rare “genomic imbalances” as the cause of their kidney dysfunction, often as part of neurodevelopmental syndromes. A new study finds that many unsuspected genetic diagnoses can be made using chromosomal microarrays to identify copy number variants (CNVs)—a “precision medicine” approach with major ramifications for treatment selection, family counseling, and long-term patient management.


Chromosomal microarrays in pediatric nephrology

The researchers analyzed the genetic findings of patients enrolled in the Chronic Kidney Disease in Children (CKiD) study—an ongoing, long-term follow-up study of risk factors and outcomes in children with kidney disease. Using patient DNA derived from stored samples, the investigators assessed CNVs using high-density microarrays.

“Chromosomal DNA microarray is a relatively new technology, which essentially looks at the entire genome of an individual and tries to identify gain or loss of DNA material that may cause a genetic disease,” Gharavi said. Microarrays represent a major advance over the microscopic technique of karyotyping—classically used to diagnose major chromosomal abnormalities such as Down syndrome. “With karyotyping, we could only...”

Dialysis Patients’ Increased Risk of Cardiac Arrest May Owe in Part to Genetics

Patients on dialysis have a higher risk of dying from cardiac arrest compared with individuals in the general population, but the factors involved are unknown. Coronary artery disease is often at play in the general population, but investigators found no significant difference in the prevalence of coronary artery disease, decreased left ventricular ejection fraction, valvular heart disease, or left ventricular hypertrophy between dialysis patients who died of cardiac death vs. those who died of other causes.

New research published in the Journal of the American Society of Nephrology now shows that the increased risk of cardiac arrest experienced by patients with kidney failure may, in part, be inherited. Uncovering the genes that are involved may point to the mechanisms underlying this risk and suggest new prevention and treatment strategies.

“It is important to stratify sudden death risk in end stage renal disease patients. The study offers a new and...”

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look at deletions or duplications larger than 1 to 2 MB. Whereas with [chromosomal microarrays], we are able to detect much smaller gain or loss of DNA material—as small as 100,000 base pairs,” Gharavi said. “As a result of this, we are able to make diagnoses that we weren’t able to make by karyotyping.”

That’s important, because small variations in copy number make up a large part of genomic variation. Even in healthy individuals, up to 10 percent of the genome may be subject to this type of variation. Previous studies using microarray techniques have identified CNV disorders associated with a broad range of congenital and neurodevelopmental defects. Genomic imbalances can affect neurologic, cardiac, and skeletal development (an effect called “ploioptery”), suggesting that common developmental pathways are involved.


These imbalances were clinically unsuspected and “overlapped significantly with CNV disorders implicated in neurodevelopmental disorders.” In the previous study, most known CNV disorders in patients with renal hypoplasia (RHD) had previously been linked to developmental delay or neuropsychiatric disorders.

Sampson, MD, a pediatric nephrologist and genetic epidemiologist at the University of Michigan, was one of the investigators in the previous study. “Understanding the underlying mechanism always helps in terms of explaining to parents why their child is ill,” he said. “From a clinical perspective it often can help in terms of providing more precise genetic testing or suggesting to us the optimal therapeutic regimen or further diagnostic tests. And, particularly important in children, it can help in terms of family counseling.”

While “personalized medicine” doesn’t always mean genetic testing, Sampson pointed out, “Genomic inquiry reveals more molecularly based, fundamental information about the pathogenesis of a child’s condition.” In regard to the new work by Verbit-Skuballa et al., he added, “This study is taking an approach that has really only recently been actualized on a clinical basis, or a research basis—to uncover some potentially causative genetic changes that could be responsible for a small but substantial percentage of the cases we see.”

**Higher rate of CNV abnormalities in children with CKD**

The analysis included 419 unrelated children from CKiD. The patients were being followed up for a wide range of clinical diagnoses, including RHD, obstructive uropathy, reflux nephropathy, and focal segmental glomerulosclerosis (FSGS), among others.

Even though CNVs account for a large part of overall variation in the genome, the population frequency of genomic disorders is very low. To be able to tell apart those low frequency genomic disorders from likely benign common variants, the researchers assembled a multibacterial dataset of 21,575 children and adults undergoing microarray genotyping for research—either healthy controls or individuals without kidney-related conditions. “By comparing the DNA of children who had chronic kidney disease to the results from these other individuals, we were able to detect rare events that could be disease-causing,” Gharavi said.

Overall, chromosomal microarrays found an average of 11 CNV disorders in 31 of the children—representing 7.4 percent of the study cohort. The CKiD cases also had a high prevalence of large, gene-disrupting autosomal CNVs: 37.7 percent, compared to 23.4 percent of the reference cohort. “This suggests that patients older than 14.3 percent of the pediatric CKD cases might be attributable to a CNV of 100 kb or larger,” the researchers wrote.

In an analysis focusing on a list of 131 known genomic disorders, 4.5 percent of the CKiD population had a deletion or duplication that was “clearly diagnostic.” These patients had a deletion or duplication with a known association with a specific syndrome. The rate of known genomic disorders rose to 10.5 percent in children clinically diagnosed with RHD.

Further annotation identified another 12 patients with a “likely pathogenic imbalance,” representing 2.9 percent of the CKiD group. These children had very large, very rare chromosomal abnormalities that were predicted to be pathogenic. These lesions were very strong criteria for pathogenicity and would be considered reportable in a clinical setting,” the researchers wrote.

Many of the detected CNVs involved genes thought to be involved in kidney development and thus may be “novel candidate genes” for kidney disease. Although previously unknown, these abnormalities are considered likely to be disease-causing because of their large size and low frequency in the population and because they involve genes important for normal kidney development.

On adjusted analysis, the odds of known genomic disorders were more than 10 times higher in the CKiD cohort overall, and 30 times higher in those with RHD, as compared to controls. Even after exclusion of known disorders (19 cases), a number of “large, rare gene-disrupting CNVs” were found in the CKiD cohort—in children with CNVs larger than 500 kb.

Close to one-fourth of patients with known or likely pathogenic copy number disorders also had rare, gene-disrupting second-site CNVs. That was consistent with reported series of patients with developmental disorders.

Most baseline clinical and demographic characteristics were similar for children with and without pathogenic CNVs. There were “nominal” differences in estimated glomerular filtration rate and proteinuria, consistent with an impact of the genetic changes on kidney function. These differences will need to be validated in longitudinal studies or independent cohorts.

**Major effects on diagnosis and clinical management**

“If you can diagnose a patient with a known genomic disorder, that might be the most important part of their kidney disease,” Sampson said. “But it also may allow us to provide additional medical care—whether it’s screening for neurodevelopmental problems, diabetes, or other congenital anomalies. It really provides the opportunity at an early stage, premanifest, to change the diagnosis—which may help to reduce the risk of long-term complications or optimize the care of a patient across their lifespan.”

In the CKiD sample, eight of 12 children clinically diagnosed with cystinosis were found to have a deletion of the cystinosin lysosomal cystine transporter gene (CTNS). For this group, CNV testing pinpointed the cause of cystinosis and provided information on the exact mutation for family counseling.

In the same cohort, 28 patients with a diagnostic copy number disorder, the final genomic diagnosis was clinically unsuspected. In these cases, the CNV findings “either resulted in reclassification of the disease or provided additional information that would have warranted genetic counseling, targeted work up, or surveillance.”

Identical genetic abnormalities were found across different clinical categories. Diagnoses of 1q21.1 recurrent microduplication were made in children with a clinical diagnosis of FSGS, hemolytic uremic syndrome, or type III polycystic kidney disease. The same XXX syndrome was diagnosed in patients classified as having recessive polycystic kidney disease, reflux nephropathy, and RHD.

“Our ability to clinically differentiate some causes of kidney disease is limited by what we can think,” Gharavi said. “Different kidney disorders can present in the same way or in many different ways that overlap with our classical classification.”

He cited the example of a patient with clinically diagnosed glomerular disorder to discuss the deeper insights offered by genetic diagnosis. “We think of glomerular diseases as something that affects just the kidney, and they’re usually due to an inflammatory or an immune-mediated disease. These types of classifications are important, because if we think somebody has an immune-mediated disease, they may be treated for it by immunosuppressive medication.”

“We are no longer having a dialog about the right medication to use, but we are starting to have a discussion about the right type of care,” Sampson said. “We can now use these data to make more tailored decisions.”

CNVs that are large are often due to problems with kidney function. In addition, these individuals are prone to developing diabetes later on in life.”

Children with renal cysts and diabetes syndrome may also have other metabolic conditions such as hypertension, azotemia or inulin clearance, hyperuricemia, or high uric acid levels, with a risk of developing gout. “The issue is that many of these complications won’t happen all at once,” Gharavi said. “The kidney cysts are evident earlier on in life, sometimes at birth, [while] the diabetes often occurs around the age of 25. Because these individuals are at risk for diabetes, they should receive targeted health monitoring to make sure that their serum glucose levels are monitored regularly.”

Patients need ongoing lifestyle advice, including control of diabetes, and should avoid medications that can potentially increase blood glucose, including immunosuppressive therapy with steroids. Other issues may arise later in life—for example, female patients should be advised that they are at risk of uterine abnormalities and problems with conception.

**Precise medicine in pediatric CKD**

By providing this type of information, CNV testing in children with kidney disease may be a prime example of the NIH’s Precision Medicine Initiative. As President Obama stated when launching the initiative, precision medicine carries the promise of “delivering the right treatments, at the right time, every time to the right person.”

In the case of pediatric CKD, chromosomal microarrays allow nephrologists to define the exact genetic diagnosis, make a profile for specific complications...
Microarrays
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very interesting idea for addressing this problem: assessing, as already done for patients with heart disease, if some hemodialysis patients possess inherited genetic factors that increase their risk of sudden death," said Simonaeta Genovesi, MD, who was not involved in this study and is a clinician and scientist at San Gerardo Hospital, in Monza, Italy. Genovesi has published many research articles related to heart health in patients with kidney disease.

For the study, Kevin Chan, MD, MSc, of Massachusetts General Hospital and Fresenius Medical Care North America, led a team that analyzed information on a population of 647,457 patients on chronic dialysis. They identified 5117 pairs of patients who came from the same family, and then analyzed which of these patients on 26 characteristics to a control patient from the same population.

The researchers found that in 4.3% of family pairs, both members died of a cardiac arrest compared with 2.6% in the control pairs. Genetically related family members who did not cohabitate had an 88% increased risk of dual cardiac arrest compared with their matched unrelated controls, while genetically related family members who lived together in the same environment had 66% increased risk.

Spouses, who were genetically unrelated but lived together in the same environment, did not have an increased risk.

"These findings advance the science because they suggest that genetic factors—or differences in DNA sequence—contribute to the high risk of sudden death among patients on dialysis," said Chan. "It paves the way for more detailed genetic studies in the dialysis population to find specific genes that could explain the high risk of cardiac arrest and potentially new treatments for these patients."

Multiple genetic variants have been identified that are linked with an increased risk of cardiac arrest in the general population. It will be important to see if these changes are also involved in cardiac arrest in the dialysis population, or whether novel variants specific to patients with end stage renal disease may explain the excess cardiovascular mortality.

Other significant factors associated with increased risk of cardiac arrest in this study included age (7% increased risk per 5 years), African American race (37% increased risk compared with Caucasian race), serum potassium level (19% increased risk per 1 mmol/L), and medical center (5% increased risk per 1000 units), and documented coronary artery disease (44% increased risk). Higher albumin levels were associated with a decreased risk of cardiac arrest.

The investigators noted that patients on dialysis have a similar risk for cardiac arrest as patients who fulfill the criteria for prophylactic implantation of a cardioverter defibrillator (ICD), but studies suggest that ICDs would not provide as great a survival benefit to patients on dialysis compared with the general population. Genetic analyses may help distinguish which patients may sufficiently benefit from ICDs.

"The study is particularly well designed, despite limitations related to the retrospective nature and the inability to do an analysis of genetic variants that may be associated with sudden death," Genovesi said. She noted that it is likely that a large proportion of the risk of sudden death in hemodialysis patients is linked to problems related to the dialysis session itself (such as hyper- and hypokalemia and acidosis) as well as comorbid conditions such as diabetes.

"I would not like to see this new attention on genetics reduce the effort made to identify the modifiable risk factors operating in end stage renal disease patients who die of sudden cardiac arrest," said Chan. "I also find it a bit risky to suggest ICD implantation for primary prevention on the basis of genetic markers in a population for which there still are several doubts on the clinical relevance of genetic intervention, as the underlying pathogenic mechanism of fatal arrhythmias in this population has not been clarified yet."

Charles Herzog, MD, an investigator at the Hennepin County Medical Center and the University of Minnesota, in Minneapolis, and was also not involved with the study, noted that the approach might make the most sense only in the prevalent hemodialysis population, which has a much lower risk of sudden cardiac death compared with newly incident patients. "In this group, I think a strong case can already be made for attempting primary prevention of sudden cardiac death, which is the rationale for the WED-HED, or Wearable External Defibrillator in Hemodialysis patients, trial," he said.

The findings come at a time when the annual mortality rate for US patients on dialysis is approximately 18 per 100 patient-years. Cardiac arrest has been reported as the largest cause, at 5 events per 100 patient-years.

Chan noted that his study’s findings are associative and only provide a promising hypothesis. Detailed genetic studies are needed to come to a definitive conclusion about the role of genetics and cardiac events during dialysis patients.

Study co-authors include Christopher Newton-Cheh, MD, MPH, James Guella, MD, MPH, and Franklin Maddux, MD.

Disclosures: KC and FWM receive salary support from Fresenius Medical Care North America.

The article, entitled “Heredability of Risk for Sudden Cardiac Arrest in ESRD,” is available at http://jasn.asnjournals.org/content/card/2015/04/16/ASN.2014090881.ab.

Increased Risk of Cardiac Arrest
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sues, educational interventions, and appropriate behavioral therapy. Behavioral issues can also affect compliance and adherence to treatment for renal dysfunction. “You can choose also your therapy for kidney disease, knowing that medications will affect neurocognitive function,” Gharavi said. “You can get a much better appreciation of the spectrum of problems that may be going on with that individual and tailor the therapy directly to their problem.”

Of course, much work remains to realize the full impact of precision or personalized medicine for children with CKD. But Gharavi emphasized that DNA microarray studies are clinically available now and are recommended as the first-line diagnostic test for children with intellectual disability, neurocognitive disorders, or major congenital abnormalities.

As these tests come into use for diagnosis of children with kidney abnormalities, Gharavi said the main challenges will be to test indications and interpretation. While many children will have a clear-cut genetic diagnosis, the situation will be less clear for the significant number of patients with other abnormal findings, including “likely” pathogenic variants. “It’s really difficult to interpret what’s causal, and what’s not, and so you need a lot more studies,” he said. Building a national research cohort of a million or more volunteers is a key component of the Pediatric Rare Disease Initiative. “There’s probably a lot of patients with neurocognitive challenges, and so there’s pretty good evidence now that [DNA microarrays] should be applied to this subset of individuals.”

“And then for the rest of the children with CKD, I think we need to expand the study and see what is the impact,” he added. The question to be answered is, “Does it make a difference to make [a genetic] diagnosis in the care of these patients?”

Sampson agreed with the recommendation to test children with kidney malformations. “With the caveat that [testing] has to be done in conjunction with the appropriate specialist who can interpret the results. Any patient who [is] sent for microarray, there should be a plan in place to also send that patient to a genetic counselor or geneticist for evaluation. Being able to properly counsel patients in terms of their genetic disorder, in terms of their risk for developing future problems or the problems they already have is nuanced and really needs help from experts.”

For now, Gharavi said RHD is the main indication for chromosomal microarrays in pediatric nephrology. “We think that children who have congenital kidney malformations are really the ones at highest risk for having chromosomal microarrays. So there is pretty good evidence now that [DNA microarrays] should be applied to this subset of individuals.”

“In addition to treatment for kidney disease, the clinical plan needs to consider treatment for neurocognitive issues—in some cases, unrelated to the patient’s kidney dysfunction—and plan clinical care accordingly.

Many of the children in the CKD cohort had pathogenic genomic imbalances associated with neuropsychiatric disorders, such as autism, schizophrenia, intellectual disability, and seizure disorders, Gharavi noted. “That’s important to be aware of, because we know that children with CKD in general have impaired neurocognitive function and behavioral issues.” Children may have problems at school and at home, or may not meet developmental landmarks.

“And many times that’s been attributed to the sequelae of kidney dysfunction and being chronically ill, being on medications, [and] being in the hospital,” he added. “We attribute this to kidney disease.”

Indeed, this group of children has a “fundamental neurodevelopmental disorder,” requiring a different approach. In addition to treatment for kidney disease, the clinical plan needs to consider treatment for neurocognitive issues and behavioral issues. "And many times that's been attributed to the sequelae of kidney dysfunction and being chronically ill, being on medications, [and] being in the hospital," he added. "We attribute this to kidney disease."

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