The New Genetics in Child Psychiatry

James J. Hudziak, M.D., AND Stephen V. Faraone, Ph.D.

In this issue of the Journal (and also in half of the issue to follow in September), we present a selection of 14 papers that cover advances in the field of child psychiatric genetics over the past 10 years. The August issue of the Journal will highlight both old and new paradigms for studying the genetics of childhood psychiatric disorders. These papers focus on candidate genes, gene-by-environment (GxE) interaction, endophenotypes, genome-wide association studies (GWAS), submicroscopic insertions and deletions, epigenetics, and genetic neuroimaging. In turn, the September issue will present papers on the advances in ADHD genetics research, ranging from twin and molecular genetic approaches, two GWAS studies, a meta-analysis of all prior ADHD GWAS studies, and a study of GxE interaction, all of which demonstrate just how far genetics research has come in child psychiatry. Some of the articles are reviews; others highlight new research. In this special edition-and-a-half, we hope that the general reader will come to better understand the importance, limitations, and possibilities of genetic research for child psychiatry.

THE HUMAN GENOME PROJECT

Before reading this issue of the Journal, some readers will benefit from a brief introduction to the field of child psychiatric genetics over the past 10 years. The first draft of the human genome was published roughly a decade ago (June 26, 2000). Shortly after the news that two drafts (albeit rough) of the human genome would be made public came promises, obstacles, and opportunities for those who practice medicine. The promises were obvious: The age of genomic medicine had arrived, and with it the long-term possibility of understanding the genetic causes of common illnesses. Optimists from outside the field of genetics even made predictions that it would be a few years until genes could be used to assist in the diagnosis of common medical conditions. Further, these discoveries were hoped to lead to an understanding of causation, which in turn, through the understanding of the functional role of the gene or genes involved in a disorder, would lead to new treatments.

The Human Genome Project delivered much of what it promised. Its maps of the genome provided a valuable tool for gene hunters, and these tools have been used to discover DNA variants that influence a wide variety of complex disorders of the cardiovascular, respiratory, immunologic, oncogenic, and reproductive systems. The same tools were used to create the International HapMap, a map of common human DNA variation that forms the methodologic backbone of much molecular genetic research. The astute reader will note our use of the term DNA variation. We use this as a general term to refer to any type of variability in DNA that might lead to disease.

Child psychiatrists saw the advances in genomic medicine as a possibility to debunk and demystify damaging misconceptions about why some children suffer with emotional behavioral illnesses and others do not. However, despite the many successes of molecular genetics, few of these dreams of child psychiatry have been realized. Genetic tests are not yet available for attention-deficit/hyperactivity disorder (ADHD), aggression, child depression or anxiety, pediatric mood disorders, autism, or obsessive-compulsive behavior. New treatments resulting from genetic research have not emerged; and sadly, many children with psychiatric illness still feel stigmatized.

However, for those of us in the field of child psychiatric genetics, the progress of the past decade has not been disappointing. Instead, programmatic research from laboratories around the world, in some cases using sample sizes in the tens of thousands, has resulted in a remarkable number of new advances that bring the field of
child psychiatry closer to the ultimate goal of understanding the evolution of child psychopathology. For the general child psychiatrist and developmental psychologist there remains the problem of just how to understand the “new genetics” of complex disorders (e.g., those conditions influenced by multiple or even thousands of genes interacting with environmental factors). Few of us received training in the new genetics, either in medical school or in residency. Yet, almost daily in newspapers, general magazines, and online, new genetic discoveries are announced. (There is even a gaming Web site named after one of the genes reported to be a risk factor for ADHD). Embedded in these discoveries is the fact that each of us needs to learn a new language, viz., the taxonomy of the “new genetics.”

What do we know? We know that the building blocks of the human genome are the four bases, adenosine, cytosine, guanine, and thymine (A, C, G, and T, respectively), which exist as matched pairs in the famous double helix that is DNA. We know that there are approximately 3 billion of these base pairs, many of which code for genes, and regulators of genes and others which are responsible for unknown, but probably important functions. We know that the human has approximately 27,000 informative sequences called “genes.” Although this is not many more genes than are harbored by the humble earthworm, human DNA has the ability to create alternative splice variants of genes, a process that creates many more proteins than genes. The invitation to discover what role genes play in human disease is the fundamental reason that we do the research we do. Just how these genes place a child at risk for psychopathology remains unknown; however, a tremendous amount of research in child psychiatric genetics has already been done, as highlighted in this special edition spanning two issues of the Journal.

Twin and Family Studies
To investigate how specific genes influence disorders, researchers follow a logical set of scientific rules. Before looking for genes that cause an illness, it is essential to find out whether that illness is influenced by genes. From twin, family, and adoption studies, we can learn whether or not an illness or trait is “inherited.” To be able to read this literature, we are often presented with new terms such as additive and dominant genetic effects, and common and unique environmental effects. In addition, equations from structural equation modeling in research reports cause many of us to “hit the snooze button,” particularly when the equations take on the appearance of hieroglyphics. These complicated statistical approaches are, of course, absolutely necessary to solve complex problems, such as those we must consider in child psychiatry.

These designs have moved us well beyond the fiery but misguided debates about nature versus nurture. We have learned that both domains affect psychopathology, exerting effects that sometimes act independently of one another and sometimes interactively, as when risk DNA variants make some children more susceptible to the onset of illness. Twin studies show that gene action can be complex, with DNA variants at a gene locus sometimes acting additively (in a dose-response manner) and sometimes with classic dominant or recessive modes of inheritance.

The environment is also more complex than early discussions presupposed. Twin studies have taught us the important distinction between environmental factors shared by siblings (e.g., social class), and those experienced by one but not both members of a twin pair (e.g., peer groups). Moreover, we now know that genetic effects are often correlated with environmental influences (e.g., a mother with ADHD who smokes will transmit ADHD risk genes and also expose her fetus to the constituents of tobacco smoke). Twin researchers have moved far beyond the computation of genetic and environmental influences and now apply twin-GWAS approaches in the study of ADHD (Wood and Neale4) and child anxiety and depression (Franic et al.5).

THE SEARCH FOR COMMON DNA VARIATION
To understand the progress of molecular genetics, readers need to understand what is meant by “common” and “rare” DNA variation and by the genetic effect size. Most extant work in child psychiatric genetics has searched for common DNA variants, by which we mean variants that occur in approximately 5% or more of the population. This focus on common variants has been mostly for practical reasons because, until recently, rarer variants have been more difficult to detect for statistical and technological reasons. However, as we will see in a subsequent sec-
tion, the search for rare variants is now also underway.

Simply put, the genetic effect size is the degree to which a DNA variant within a gene affects the onset of a disorder. At one extreme are DNA variants that account for 100% of a disorder’s prevalence. Most readers are used to the idea that “disease genes” “cause” illness, i.e., if one inherits the so-called disease gene, one becomes ill 100% of the time. For example, individuals with the Huntington disease genotype will all eventually develop Huntington disease. The same is true for other rare genetic conditions such as Tay-Sachs, and Duchene’s muscular dystrophy. For such single-gene disorders, transmitted by classic Mendelian autosomal dominant, recessive, or sex-linked modalities, gene discovery is relatively easy, as genes with such large effects can be detected with the genetic linkage analysis of large samples. It was hoped that similar discoveries, e.g., a single gene of major effect, might be found for the child psychopathologies. Unfortunately, to date that has not been the case. Although genetic linkage analysis has been applied to many child psychiatric disorders using large samples and meta-analyses, no causal DNA variant has been discovered using this method, and replications of promising linkage findings between studies has been rare. This has led to the important discovery that, if common variants exist, their genetic effect sizes must be very small. If this is true, then to explain the prevalence of psychiatric disease by common variants, one must assume that many common variants work together to cause illness.

To search for common variants having small effects, one must use the method of association. A classic association design compares patients who have a disorder with persons who do not have the disorder and asks if the patient sample is more likely to harbor any DNA variants. The most frequently used association approach has been the study of “candidate genes.” The method is straightforward: Pick a gene that seems to be a plausible candidate for playing a role in an illness or trait based on prior knowledge, typically pharmacologic response. For example, because the mechanism of action of stimulant medications has implicated dopamine (DA) in the pathophysiology of ADHD, genes that code for proteins in DA signaling pathways are candidate genes for ADHD. Similarly, many papers examine DNA variants in the serotonin transporter gene as a risk factor for mood, anxiety, and post traumatic outcomes given the efficacy of serotonergic drugs for these disorders. Candidate gene studies have been successful in the sense that many have been completed and, when multiple data sets have been analyzed, meta-analyses have suggested that specific DNA variants are involved in the etiology of disorders. Two well-known examples are positive meta-analytic results for the dopamine transporter and D4 dopamine receptor genes as being associated with ADHD.

However, when significant, gene–behavior relations have typically accounted for the identification of very small amounts of the total heritability of a disorder (approximately 1-2% of the genetic influence). For instance, while twin studies report that ADHD is influenced 60% to 90% by genes, at best, only 5% of that influence can be traced to specific candidate genes. In some ways, this discovery was also an important one because it motivates us to think of genes in a different light. Rather than think of genes as disease genes, which they almost certainly are not, it is perhaps better to embrace the concept of susceptibility genes, i.e., that some DNA variants increase the risk for the brain variations underlying a disorder but are not necessary and specific causes of the disorder. In this way, each DNA risk variant can be seen as being responsible for influencing a trait, expressed as a variation in brain functioning, and the combination of these traits may be what puts one child at risk for a disorder, and yet a different combination may be protective. Albaugh et al.6 present a compelling example of this line of thinking. Following a well known physiologic hypothesis, the various forms (alleles) of the catechol-O-methyltransferase gene (COMT) are hypothesized to regulate dopamine metabolism, to thus influence tonic and phasic firing rates in the prefrontal cortex. Albaugh et al. posit that this chain of events places children at risk for inattention or aggression.

Candidate gene studies have advanced to considering the role of the environment in a child’s outcome. In this issue, we have two papers on GxE interaction. One, a review by Nigg et al., provides readers with a clear understanding of GxE approaches and reviews prior ADHD candidate gene studies, prior studies on environmental influences on ADHD, and prior studies addressing the interaction between the two.7 It probably will not be surprising to understand that a child’s environment, if he was raised in
adversity or if his mother smoked during pregnancy, will play a role in his neurodevelopment. The second paper, reporting new research by the Moffitt and Caspi group, presents a story of a preventable gene-environment interaction. In it, Sugden et al. provide a chilling report about how children with variation in the 5-HTTLPR variant of the serotonin transporter gene are at risk for developing emotional and behavioral problems if they were victims of bullying. Specifically, in a study of 2,322 British children, those carrying the SS variant and were bullied were at greater risk to have emotional problems at age 12 years. Candidate gene studies such as these could lead to public health interventions (e.g., greater emphasis on smoking cessation programs for pregnant women; greater efforts to decrease bullying) that may lower the prevalence of child psychopathology.

As a result, candidate gene studies are useful in identifying GxE interaction, and in understanding the role that common DNA variants may play in pathophysiology, even though they may never explain the overall genetic architecture of an illness (because, by themselves, they explain such a small percentage of the inheritance of any disorder). Some experts believe that, if common DNA variants play an important role in child psychopathology, their discovery may require us to attend more carefully to GxE interaction, a hypothesis that is consistent with the failure of candidate gene studies to easily explain the heritability of childhood-onset disorders.

GENOME-WIDE SEARCHES FOR COMMON DNA VARIATION

The search for GxE interactions is only one method for improving the odds of discovering common DNA variants. Another approach is the genome-wide association study (GWAS). Instead of restricting its search for common DNA variants to candidate genes, GWAS interrogates the entire genome. This has been made possible by improved methods (both molecular genetic and statistical), and the creation of large, collaborative, multinational samples. GWAS searches the entire genome using 500,000 to 1+ million DNA variants known as single nucleotide polymorphisms (SNPs) that are selected in a manner that allows researchers to test gene–disease associations with nearly the entire genome. The articles in the special issue of the journal use a variety of sampling approaches (trio, family-based, and case-control designs) across several disorders and traits (ADHD, obsessive-compulsive disorder, Tourette syndrome, and learning abilities and disabilities). GWAS determines whether any SNP is statistically associated with any given disorder. In a trio of new research publications on ADHD, the reader can learn a great deal about GWAS approaches, and the importance of taking a wide variety of strategies when looking for genes. Two of these studies report new GWAS data from two independent consortiums. The IMAGE II Consortium used a case control design, and a consortium data set reported by Mick et al. used a family-based design. In a third paper, the ADHD GWAS Consortium used meta-analysis to combine GWAS results from these two studies and two additional studies to create a remarkable sample size of 2,064 trios, 896 cases, and 2,455 controls, making it the most informative genetic study of ADHD ever completed. Although these GWAS do not report genome-wide significant findings, they each teach us a great deal about the promise and limitations of GWAS and provide further hope to the field with a concrete example of how many investigators can cooperate to create large, genetically informative samples.

Grados provides us with an up-to-date review of genetic approaches, including GWAS, to the study of OCD and Tourette syndrome (TS) that cogently describes alternative methods for discovering common DNA variants. These include the use of newer diagnostic/phenotypic identification approaches, intermediate phenotypes (endophenotypes), and the study of gene networks and neural systems to discover the genetic etiology of OCD and TS. Haworth and Plomin further elaborate the point on Grados by beautifully pointing out that genetic research is quantitative research, and that common disorders are best to be viewed in a variety of ways, e.g., by considering that common disorders are quantitative traits, or by considering the generalist gene hypothesis, and finally the nature of nurture hypothesis in their review of learning abilities and disabilities.

The discriminating reader might point out that none of the papers appearing in this issue of the journal report positive GWAS findings. However these negative findings send a valuable message: If common DNA variants cause child psychiatric disorders, their individual effects must be very small.
For example, no single variant may increase the risk for illness by more than a few percentage points. It is important to note, however, that just 5 years ago there was not a single positive GWAS finding reported for any medical condition. The first, late in 2005, was for age-related macular degeneration. In the 4.5 years that have followed, GWAS have led to more than 600 reported findings of genome-wide significance across a wide range of medical disorders. Positive GWAS findings have recently been reported for schizophrenia and bipolar disorder using much larger samples than are currently available for child psychiatric disorders. We can only hope that funding agencies will provide similar funding levels to the investigation of child disorders so that similar sample sizes can be achieved.

WILL RARE VARIANTS SAVE THE DAY?
Although the failure of GWAS to deliver positive results may be due to small samples, some authors have argued that the search for common DNA variation is misguided; they suggest that rare variants account for much of the familial transmission of psychiatric disorders. The search for rare variants is proceeding on two fronts. Fortunately, although GWAS had been originally designed to search for common variants, most GWAS technologies also produce information about deletions and insertions in the genome, which are known collectively as copy number variants (CNVs). CNVs are essentially submicroscopic cytogenetic abnormalities. Many studies have documented how very large insertions, deletions, and chromosomal rearrangements lead to child psychopathology; velocardiofacial syndrome is one example. But do the much smaller CNVs known to exist in the genome contribute to child psychopathology? The short answer is yes. Published studies have already implicated CNVs in ADHD and autism. In this special issue of the Journal, Hoffman and State provide us with a complete review of the study of chromosomes and CNVs as they relate to developmental psychopathologies such as ASD, childhood-onset schizophrenia, and Tourette syndrome. This fascinating paper reveals the important finding that not only the sequence of the DNA but also the fine structure of the chromosomes may play a role in causing developmental psychopathology.

It is too soon to know whether rare variants will save the day. The CNV studies are promising, and it has very recently become feasible to sequence the entire human exome (i.e., the fraction of the genome that codes for proteins). This new method makes it possible to discover rare causal SNPs throughout the exome. The 1000 Genomes Project will soon provide researchers with many rare functional SNPs to study as will the full exome scans currently underway or in the planning stage for child psychopathology. We are likely soon to discover rare SNP variations implicated in some cases of our more common disorders.

BEYOND DNA: EPIGENETICS AND FUNCTIONAL GENOMICS
Leaving the genome, we move to two papers that address epigenetics, the study of cellular events that modify the process whereby genes build proteins. Unlike mutations, which directly change the genetic code by deleting, inserting, or changing bases, epigenetic events change the amount of protein produced and, sometimes, how it functions. Epigenetic events are crucial because they provide the biological basis for how environmental risk factors change the expression of genes to modify cellular functioning. Most epigenetic studies focus on one of two mechanisms: methylation and histone acetylation.

A simple introduction to the epigenetics, believe it or not, is possible. Keeping in mind the four bases (A-C-G-T) and that A pairs with T and C pairs with G in the alpha helix, all you need to know is how they are bound. For simplicity, a phosphate group (p) binds each pair. Thus a C-p-G island is simply a CG pair somewhere in the genome. If the C-p-G island happens to be in the promoter region of a gene, it is a prime candidate for an epigenetic event. Methylation is the classic epigenetic event discussed in two papers in this issue. You will remember the methyl group from organic chemistry as simply a carbon surrounded by three hydrogen molecules (CH3). Epigenetic methylation is the event by which a CH3 binds to the “p” of a C-p-G island. Such events cause genes to be over- or underexpressed. In the paper by Bagot and Meaney,15 the reader will learn in detail the entire process of epigenetics (both methylation and histone acetylation). Here the reader will learn how the environment can lead to gene function being altered without the structure of genes themselves being changed. This paper helps us understand one biological mechanism whereby the environment
regulates the genome and exerts its effects on developmental psychopathology. Grafodatskaya et al.\textsuperscript{16} then present a detailed review of how epigenetics may be associated with the development of autism spectrum disorders (ASD). These epigenetic studies are moving the field towards the modeling of eGxE traits (a model in which the environment affects the epigenome, which then interacts with the environment, which in turn effects the epigenome, and so forth).

As we have described above, the discovery of common and rare DNA variants that increase susceptibility to child psychiatric disorders will require large international collaborations and sophisticated molecular and statistical technologies. Yet, the discovery of confirmed DNA variants is merely one step on the pathway to discovery. Much more work must ensue to determine the functional effects of DNA variants and how this information can be used for the discovery of new treatments, both pharmacologic and environmental. The term “functional genomics” refers to paradigms that study the functional effects of DNA variations. Much current work evaluates how DNA variation affects the expression of messenger RNA (mRNA) in relevant tissues. Proteomic studies assess the degree of protein expression and the structure and function of mutant proteins. In the study of child leukemia, it was not the study of DNA but rather mRNA and proteins that led to remarkable advances in identifying pathophysiology and developing effective treatments that dramatically reduced mortality. The success of the study of mRNA and protein expression in a leukemia cell directly led to the understanding of the illness. Similar advances have been made in other cancers; in each case, the cell studied was the one in which the disease is manifest. Ideally, psychiatric geneticists would do parallel studies using brain tissue from psychiatric patients. Although there are brain tissue resource centers for adult-onset disorders, child psychiatric studies rarely have had access to brain tissue. Thus such studies are difficult to perform or must rely on peripheral tissues or animal models, both of which raise issues of generalization.

As a result, many researchers think that functional genomic studies in child psychiatric illness are at an impasse. Pine et al., however, show how neuroimaging genomic studies can break that log jam.\textsuperscript{17} These authors show how we can combine neuroimaging and genetic paradigms to better understand genetic contributions to the structural and functional brain anomalies that have been observed in many child psychiatric disorders. Given the complexities of the human brain, studying function and structure in vivo may well be superior to post-mortem and animal studies. Only in psychiatry can we combine neuroimaging and genetics in a way to ask the simple question: Do individuals with different DNA variants or epigenetic events have differences in brain structure and function? As Pine et al. write, “The approach holds promise for advancing the understanding of pathophysiology and therapeutics.”

PUTTING IT ALL TOGETHER

Only 10 years ago, the first draft of the human genome was reported. In the intervening period, genetic research on developmental psychopathology has grown exponentially, as reflected not only in the number of published papers but also in the power of molecular genetic and statistical technologies. Although we are only in the infancy of our field, the pathway to discovery is clear. One can only imagine the incredible progress that will be made in the next decades. It is our hope that this special issue of the Journal will stimulate your interest in learning all that you can about the “new genetics in child psychiatry.” At the very least, understanding the complex interplay between genes, the environment, and the epigenome will help you to understand why some of your patients struggle so mightily; and perhaps, by using this new information, you can work hard to help them change their eGxE.
REFERENCES