Unraveling the Genetic Contributions of Cognitive Control

Neuroscience researchers are using the NovaSeq™ 6000 System to identify variants for the development of a new sequencing array designed for ADHD, autism, and schizophrenia studies.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a common behavioral disorder in children and adolescents throughout the world. Experts estimate that approximately 2–7% of the childhood population have been diagnosed with the disorder. These experts also believe that the condition remains remarkably underdiagnosed, particularly in females and adults.[1] A better understanding of the genes that contribute to ADHD could lead to the identification of ADHD-associated biological risk pathways. This knowledge could become the foundation of diagnostic tests that enable clinicians to identify children at risk of developing the disorder earlier and inform innovative personalized treatment paradigms.

Mark Bellgrove, PhD, is the Director of Research at the Monash Institute of Cognitive and Clinical Neurosciences, and a professor in cognitive neuroscience in the School of Psychological Sciences at Monash University. His work focuses on defining the molecular genetic architecture and neurochemical drivers underlying two key components of executive function: attention and cognitive control. By taking a systems neuroscience approach, Dr. Bellgrove hopes to identify the genes that contribute to ADHD, and build an understanding of the cellular, brain, and cognitive pathways that lead to the disorder's behavioral symptoms.

Dr. Bellgrove is working closely with Illumina to design a customized array, the CogChip, to identify variants associated with executive function and cognitive control issues. He spoke with iCommunity about how rare genetic variants might increase our understanding of executive function, why whole-genome sequencing (WGS) is the future for investigating complex disorders, and how next-generation sequencing (NGS) could herald the advent of personalized ADHD treatments and therapies.

Q: What originally interested you in studying ADHD?
Mark Bellgrove (MB): I've always been interested in the biology of cognition. For example, you and I might be different in our ability to focus and pay attention. Why is that? Why are some people easily distracted, while others can remain focused even in distracting situations?

My training was in cognitive neuroscience, looking at the neuroscience behind attention and working memory. While in a postdoctoral fellowship at Trinity College in Ireland, I started to work on ADHD, performing some of the first studies linking gene variation in the Irish ADHD population to laboratory measures of attention. That sparked my interest in aligning my cognitive neuroscience work with a genetic approach.

Since then, my work has expanded to a systems neuroscience approach to understanding ADHD. I'm trying to understand how genes and gene mutations might alter cell signaling pathways, and how that cascades from a physiological response in the brain to some objective problem of attention or cognition in ADHD children.

Q: As someone who has studied ADHD in different countries, what is the prevalence of the disorder is Australia as compared to other Western countries?
MB: Surprisingly, the prevalence is pretty uniform across the globe. Population-based epidemiological samples suggest that ~5% of school-aged children have ADHD.[2] Despite some of the headlines you might read, ADHD diagnosis rates are consistent no matter where you are in the world and haven't changed significantly over the last few years.

Q: What do we know about the causes of this disorder?
MB: We know that ADHD is one of the most heritable of all mental health conditions. The percentages of individual differences driven by genetics are similar to what is seen in other disorders, such as autism and schizophrenia. We know that ADHD is driven by genetics, however we're still trying to isolate all the gene variants that might confer risk.

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There are common variants (variants that exist in the general population), which amount to a minor allele frequency of about 5%. However, rare genetic events that damage gene function might also play an important role. We're using NGS to identify rare mutations that might have a significant impact on gene function. Similarly, autism research has used sequencing to uncover variants that impact functional pathways in the disorder.

Q: What methods have been used to study the ADHD genetics?
MB: Like many complex disorders, we started looking for so-called candidate genes. These were the genes that we thought might be involved in the etiology of ADHD based on a biological hypothesis. For example, we know that stimulant medications treat ADHD effectively and work by inhibiting the dopamine transporter protein in the brain. This protein is responsible for the reuptake of dopamine from the synapse. One biological hypothesis was that this transporter was overactive. Researchers began looking for gene variants that encoded the dopamine transporter to see if there were unequal frequencies between ADHD cases and controls. Those studies, at least for the dopamine transporter and for a dopamine receptor called D4, suggested that those candidate genes do confer a small amount of risk. However, a criticism of these studies was that they cherry picked the genome for specific genes *a priori*, but didn't provide a view of the genetic risk for ADHD in the context of the whole genome.

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To look at the whole genome, researchers turned to genome-wide association studies (GWAS). For a long time, GWAS were remarkably unfruitful for ADHD due to the small sample sizes. However, the Psychiatric Genomics Consortium and other international cohorts have expanded the number of international ADHD datasets. Today, there are 14 meta analysis–verified GWAS gene hits for ADHD.[3] However, these studies only address common variants that have a minor allele frequency of less than 1% or 5%, depending on the specific criteria used. These studies have not addressed rare variants and how they might impact gene function.

Q: Why did you begin using NGS in your studies?
MB: NGS enables us to view the entire genome and identify rare variants in coding and noncoding regions efficiently. We're using WGS and whole-exome sequencing (WES) in our studies.

Initially, we performed a small WES study with a targeted gene set derived from the literature and found evidence of rare variants within the brain-derived neurotrophic factor (*BDNF*) gene, an important gene for brain development, in ADHD cases compared to controls.[4] That was a small, preliminary study that we want to replicate in greater detail.

Q: What are executive functions and how are these processes altered in ADHD?
MB: Executive functions refer to uniquely human cognitive processes that enable us to interact and adapt to the complex, ever-changing world in which we live. Executive functions are a group of cognitive processes that allow you to attend to what is important and to inhibit behavior when it's not appropriate, whether it's inappropriate internal thoughts or outward actions. These kinds of cognitive functions are impaired in ADHD, as well as in schizophrenia and autism. By studying the genetics of executive function, we might uncover new clues to the underlying genetic liability for ADHD, autism, and other disorders.

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Q: What are the objectives of your executive functions research?
MB: We know that executive functions are highly heritable from twin studies. If you measure monozygotic twins, executive functions are more similar than what you see in dizygotic twins. I partnered with Illumina to look at a large cohort at Monash University of healthy individuals who have been screened or assessed on executive function measures. We're using GWAS and WES to find both common and rare variant associations for individual differences in executive functions. Ultimately, we'll need to perform very large studies for this research to be successful.

Partnering with Illumina gives us the opportunity to explore the genetics of executive functions. If we can find gene variants that relate to individual differences, then we can develop a targeted chip that is enriched for various genomic regions and potentially use it as a screening tool for a range of conditions marked by executive control problems from ADHD to schizophrenia.

Q: Have you begun NGS studies to identify variants that are associated with executive functions?
MB: We just initiated a WES study in 2018 using the NovaSeq 6000 System. We are currently waiting for the sequencing data to be returned to us. We're using a Monash University databank comprising DNA samples and detailed phenotypic information from 1200 healthy, young adults that will be used as normal

controls, together with a collection of 1800 samples of children affected with ADHD and their parents (600 trios).

In parallel, we're also performing consortium studies and have developed a lab-based protocol to assess how well someone can inhibit their behavior when it's inappropriate or unwanted. With an international consortium, we're performing a GWAS of that trait to see if we can identify the genetic substrates of response inhibitions. With around 10,000 samples from that consortium, we have a real potential to show something interesting.

Q: Why did you choose to use WES instead of WGS for this initial study?
MB: WGS will be the desired platform for ADHD studies because we believe there's a strong contribution of noncoding variants in these disorders and we can survey noncoding variants better with WGS than with WES. However, our current protocol is driven by cost constraints. Currently, WES is more cost effective than WGS. The method we are using has exome capture as well as some capture of noncoding regions such as the intron–exon boundaries and untranslated regions.

As costs come down and the methods improve, WGS will become an efficient, cost-effective approach for studying ADHD and other disorders where we believe that noncoding regions are critically important. The ENCODE project has shown that noncoding variants are likely to be relevant to a whole host of complex traits.

> "We believe the CogChip could be the basis of a screening tool, because executive functions are a useful proxy for a wide range of different clinical disorders."

Q: How will WES and WGS integrate into your systems neuroscience approach?
MB: NGS is an important element of our systems neuroscience approach to ADHD. Part of what attracted me to NGS was the possibility to identify causative mutations and then put those into cell lines to perform functional validation. We have just started creating induced pluripotent stem cells (iPSCs) for ADHD. We hope to use these cells to engineer mutations that we find as being likely causative for ADHD into the iPSCs, then differentiate these into neurons. We have a biological hypothesis that dopamine neurons are dysfunctional for ADHD, but we don't actually have any objective evidence to show that. iPSC-derived neurons are a valuable way to take that hypothesis and test the impact of genetic mutations on dopamine signaling.

From now on, our desire is to develop mechanistic accounts of ADHD pathology. That's why I'm using sequencing. We can identify mutations that are immediately tractable to functional validation using iPSC technology. We can see a variant's impact on cell function. Then we can look for evidence that this gene alters structural, functional, or connectivity patterns within the brain, as well as look for objective problems of executive function. A systems neuroscience approach will help us close the loop between genetic risk and the actual ADHD phenotype we see in the clinic.

Q: What variants will be included on the CogChip and how will it support ADHD studies?
MB: Our current studies will inform construction of the CogChip. We anticipate that the CogChip will have a common variant backbone and will be enriched with rare variants that we identify from our sequencing protocols, as well as variants identified in large consortium studies as being associated with brain or cognitive function. I imagine it will be similar to the Illumina PsychArray, except that it will be enriched with variants that we see broadly in executive function and brain functions.

> "Our work has the potential to inform personalized medicine approaches based on genetic variant associated stratifications."

Q: How could the CogChip be used to inform the development of ADHD diagnostics and personalized treatments?
MB: We believe the CogChip could be the basis of a screening tool because executive functions are a useful proxy for a wide range of different clinical disorders. We could potentially use it to screen for genetic liability for whatever trait we are interested in studying. It could also be useful for early detection and diagnosis, as well as to stratify populations to study potential drug treatments or personalized medicine strategies. There could be a broad range of applications.

Q: How will your studies impact our understanding of ADHD?
MB: Diagnosis for ADHD is subjective. We know it's a massively heterogeneous condition, with many comorbidities and subtypes. Unfortunately, data from past GWAS grouped all individuals with ADHD as though they had the same form of the disorder. It assumed that they had similar underlying pathophysiology, which almost certainly isn't true. A benefit of our ADHD genetic studies will be to see if there are clusters, symptoms, or cognitive behaviors that are linked to structural or functional brain abnormalities. This information would support the stratification of cases and potentially identify unique genetic signatures for distinct subtypes of the disorder. That could, ultimately, aid in diagnosis.

Currently, clinicians have nothing at their disposal to tell them whether a child will respond to a medication or not. As a result, they prescribe ADHD medications on a trial and error basis. Our work has the potential to inform personalized medicine approaches based on genetic variant associated stratifications. For ADHD, the effectiveness of treatments like methylphenidate, or Ritalin, might vary by polygenic risk. Genetic screening could identify those risks.

Q: How do you see the field of cognitive neuroscience capitalizing on the power of genomics?

MB: By understanding the genetics of executive function and the distinct aspects of cognition, irrespective of a particular clinical pathology, we can better understand individual differences in thinking and the circuits in the brain that mediate those differences.

By building on these mechanistic paths of cognition, we can better understand what's going awry with different clinical disorders. I don't see these two approaches as being mutually exclusive. I think they can reinforce one another, feeding into and back on to one another.

Sequencing will be a powerful tool to help us better understand brain function. We're the first to use NGS for studies of executive function or ADHD and I'm very excited about what it might help us uncover.

Learn more about the products and systems mentioned in this article:

NovaSeq 6000 System, www.illumina.com/systems/sequencing-platforms/novaseq.html.

References

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