# Multiple Rare Gene Variants May Break Autism's Silence

In the most comprehensive DNA-autism study to date, the Infinium® Human1M BeadChip identifies rare genetic alterations in genes involved in brain cell communication, cell growth, and motility.

## Introduction

Autism is a disease of extremes, encompassing highly intelligent and functioning individuals, as well as those with severe mental deficits and often profound impairment in social interaction and communication. It forms the core of autism spectrum disorders (ASDs), a number of presumed neurodevelopmental conditions that afflict one in 110 newborns[1] and 1% of U.S. children between the ages of three and seventeen[2].

For years, there were no answers as to what caused these disorders or why they affected some children and not others, leading to widespread speculation of environmental causes such as pollution or the use of childhood triple vaccine for measles, mumps, and rubella (MMR). While environmental factors have not been ruled out, the data implicating the MMR vaccine was recently discredited[3].

What is known is that ASDs are highly inheritable, often running in families. Several autism-linked genes have been identified, and while some rare single genes appear sufficient to cause autism, in the majority of instances it is likely different gene permutations impact not only the presence of ASD, but also where along the spectrum of disorders a child's case may fall.

### The World's Largest ASD Genetic Repository

In the past, ASD genetic studies were limited by small sample sizes, usually consisting of family groups that provided a limited view of the autism spectrum. Recognizing that the varied ASD phenotypes could be caused by an equal number of autism-linked gene combinations, researchers around the world decided to join forces. They needed a larger repository and database of well-characterized clinical material, the financial resources to perform the necessary whole-genome scans, and a genotyping platform to standardize the genome scans to generate high resolution data.

The result was the Autism Genome Project Consortium, which united 150 scientists from 11 countries behind the goal of improving scientific collaboration and accelerating the pace of scientific discovery into the genetic causes of ASD. Stephen Scherer, Ph.D. and his team at The Center for Applied Genomics at Toronto's Hospital for Sick Children were founding members of the consortium, and participated in the selection of the Illumina Infinium Human1M BeadChip as its genotyping platform. "At the time, we were testing the hypothesis that common SNPs would have a role in autism and wanted to use a genome-wide association study (GWAS) approach," said Dr. Scherer. "Illumina's history of high SNP genotyping calls, tipped the scales in its favor. At the same time, we were starting to realize the importance of also capturing copy number variation (CNV). Ultimately, it turned out that the 1M BeadChip excelled in both areas."

Since it was founded in 2004, the consortium has collected genetic data and clinical information from more than 3,000 autism families worldwide. "This database is now supporting the analysis of thousands of patient samples to determine the role of rare copy number variations in ASD," according to Dr. Scherer. "I believe this is one of the largest ongoing genomic studies of any type being ever undertaken."

### Rare Variants May Thwart Brain Cell Communication

A member of the consortium's executive committee for international projects, Dr. Scherer is also the Canadian team co-leader on the rare variant autism project. The study is being conducted in two phases, with rare CNV analysis of the first 1,275 ASD cases and their parents completed in 2010[4]. A set of 1,981 controls was initially used for comparison, with both sets genotyped on the 1M BeadChip and subjected to the same quality control procedures. Ultimately, both groups were trimmed down, with 996 ASD cases and 1,287 controls of European ancestry analyzed to minimize confounds due to population differences. To assess the impact of rare CNV in cases compared to controls, three primary measures of CNV burden were used: the number of CNVs per individual; the estimated CNV size; and the number of genes affected by CNVs.

While there was no significant difference found between cases and controls in the first two measures, there was a significant increase in the number of genes intersected by rare CNVs in autism cases. This ASD association with genic CNV was stronger for deletions, particularly with single-occurrence CNV deletions, indicating that some could be pathogenic.

Stephen Scherer, Ph.D. is Director of The Center for Applied Genomics at Toronto's Hospital for Sick Children, Director of the McLaughlin Center at the University of Toronto, and a member of the Executive Committee of the Autism Genome Project Consortium.

"There were 100 or more different genes affected by autism CNVs that were either not seen in controls or existed there at a much lower frequency," said Dr. Scherer. "While numerous interesting ASD candidate genes were found, in particular four of them stood out, including SHANK2, DDX53-PTCHD1, DLGAP2, and SYNGAP1, because they were found as CNVs in multiple families. We performed more detailed molecular analysis of these novel candidate ASD loci and found they encoded proteins involved in brain synapse development."

The human brain contains millions of neurons, each linked together with up to 10,000 synapses. In the cerebral cortex alone, one cubic millimeter contains roughly one billion synapses[5]. Copy number variations in ASD loci could be responsible for gene dosage alterations that alter protein homeostasis critical for synapse development, ultimately impairing how neurons communicate with each other and impacting an individual's intellectual capacity, behavior, and ability to communicate.

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Further molecular analyses showed that SHANK2[6], which is related to the SHANK3 gene previously shown to be an ASD-linked locus, and DLGAP2 are more involved in neurotransmitter connections and synapse formation, with SYNGAP1 impacting intelligence. Deletions at DDX53-PTCHD1 were also found to be strongly associated with ASD and intellectual disability[7]. Ultimately, researchers identified a total of 226 validated *de novo* (7) and inherited (219) CNVs affecting single genes found in ASD cases.

### Deletions Over-Represented in Global CNV Burden Analysis

"The real power of this study is the comprehensive analysis we performed to create the highest quality CNV data set in autism or really for any disease so far attempted," said Dr. Scherer. "It allowed us to perform a global CNV burden analysis, systematically looking across the genome of ASD cases versus controls to test if the CNVs, whether deletions, duplications, or repetitive elements, were over-represented in ASD cases. The only feature we saw that was over-represented were deletions in exons of genes, suggesting that if you carry more CNV deletions in specific genes you will be at higher risk of developing autism."

Genes such as SHANK2 and SHANK3 were highly penetrant, meaning that if a deletion occurs in one of these genes, ASD or intellectual disability will likely result. Deletions on the DDX53-PTCHD1 gene were the exception, with a higher incidence in cases over controls, yet still appearing in some female controls. That is because the gene is maternally inherited, residing on the X chromosome. Female controls could carry a deletion in one copy of this gene, yet are phenotypically normal, because they possess a protective X chromosome.

"This starts to explain why there is a higher frequency, about 4:1, of autism in males than in females," said Dr. Scherer. "Females can be carriers and pass CNV deletions in the X chromosome PTCHD1 gene to their male children, who will then be on the autism spectrum."

What still is not yet understood is the impact of environment, i.e. all causes other than genetics. "We know that genes such as SHANK2, SHANK3, PTCHD1, and others together account for about 15% of the genetic variants in autism. Yet, we anticipate that genetics will have some role in 90% of autism cases, because in identical twin studies, if one child is autistic, the second child will be on the spectrum in 90% of the cases. So what genetic errors are causing the other 75%? Our findings support the involvement of multiple rare genic CNVs in ASD, both genome-wide and at specific loci. Clearly, ASD-linked CNVs and other genetic changes exist that we still need to identify."

### Study is Sparking ASD Diagnostics and Therapeutics Development

Currently, only the concomitant symptoms experienced by ASD individuals can be treated. Anxiety, sleep disturbances, and metabolic and gastrointestinal issues can be alleviated with drugs that are currently on the market, providing some ASD individuals with a better quality of life and an opportunity to live at home. However, assays and therapeutics designed specifically for diagnosing and treating ASD have yet to be developed.

The consortium's CNV research could dramatically change the diagnostic and treatment paradigms for these disorders. It has already sparked interest from molecular diagnostic companies, with several using the data as the basis for microarray screening to support autism diagnoses, while others are developing new DNA-based assays for SHANK2, PTCHD1, and other targets. On a longer term horizon, drug companies are investigating the ASD-linked connected pathways uncovered by the research, mining them for proteins or other targets that could be used to develop more effective therapeutics.

"With the help of the 1M and 1M-Duo BeadChips, we have been able to generate and publish high quality data on ASD rare variants that researchers will be able to use for years to come."

Armed with the largest autism genetic repository in existence, the consortium is proceeding with the next phase of the project using the Illumina 1M-Duo BeadChip to perform genotyping studies of another 2,000 families. "With the help of the 1M and 1M-Duo BeadChips, we have been able to generate and publish high quality data on ASD rare variants that researchers will be able to use for years to come," Dr. Scherer said.

The consortium continues to provide added value, enabling researchers to easily investigate how the variants they identify are segregating in families. What once would have required contacting multiple clinical sites to capture information or perform more clinical testing, can now be completed quickly and efficiently through the established international consortium network.

“Consortium researchers in Canada and the United States are now also sequencing the exomes, and ultimately the genomes, of the thousands of ASD cases and controls,” Dr. Scherer said. “I think the exome data will tie in very nicely with our CNV data, further revealing the still to be discovered genetic forms of ASD.”

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