

Genetics in Autism Diagnosis: Adding Molecular Subtypes to Neurobehavioral Diagnoses

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INTRODUCTION: AUTISM GENETICS RESEARCH HAS DIRECT RELEVANCE TO THE CLINIC

Autism is a heterogeneous neurodevelopmental disorder that affects people of all races, ethnicities, and backgrounds and is about four times more prevalent in males.¹⁻³ The incidence of autism has risen so dramatically over the past few decades that this increase has been termed the “autism epidemic.” A recent surveillance study reported a 57% average increase in the number of autism diagnoses in specified regions of the United States from 2002 to 2006, and estimated the current prevalence to be one in 110 children.⁴ The increase in diagnosis can be at least partly attributed to greater awareness, broader diagnostic criteria^{5, 6} and improved services for autistic children.⁷ Regardless, more children than ever are in need of proper diagnosis, treatment and services for autism.

This article focuses on recent progress in genetic studies relevant to autism diagnosis. This progress has highlighted the genetic heterogeneity of autism, which mirrors the variation in clinical presentation of behavioral symptoms. Genetic research has recently revealed that about 10% of autism diagnoses can be subtyped according to genetic abnormalities.⁸ Genetic testing has, therefore, entered the clinical arena. As the multiple genetic etiologies of autism continue to be elucidated and as molecular genetic testing becomes more widely available and less expensive, genetic subtyping of autism will become more common.

DIAGNOSING AUTISM: THE UTILITY OF GENETIC SUBTYPES

In the United States, autism is primarily diagnosed on the basis of characteristic behaviors outlined by the American Psychiatric Association in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR).⁹ These behaviors include aberrant social interaction, impaired ability to communicate through both verbal and

nonverbal means, repetitive behaviors, and obsessive tendencies or fixations. DSM-IV-TR lists five disorders under the umbrella term Autistic Spectrum Disorders (ASDs): Autistic Disorder, Asperger's Disorder, Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS), Rett's Disorder, and Childhood Disintegrative Disorder (<http://www.cdc.gov/ncbddd/autism/hcp-dsm.html>). These disorders are distinguished by age of onset, specific behaviors, and severity. Rett's Disorder is the only ASD with a known genetic cause: it is an X-linked, Mendelian disorder caused by mutations in the *MECP2* gene.¹⁰

Because diagnosis of autism is based on clinical symptoms, a diagnosis does not necessarily reveal information about biological cause. Furthermore, clinical symptoms alone may not be sufficiently informative to guide prognosis or treatment stratification. Genetic testing is now linking behavioral diagnoses to genetic loci. Distinct genetic subtypes provide a greater degree of biological explanation for patients and families, and as such may facilitate identification of medical co-morbidities or clinical outcomes. Additionally, diagnostic genetic tests are of great benefit because they may allow for earlier, more accurate detection of autism in young children. Data suggest that the earlier a child begins treatment for his or her autistic symptoms, the greater the chance that the symptoms will diminish;⁵ however, it can be difficult to make a clinical diagnosis earlier than two years of age because many characteristic behaviors are related to the acquisition of language and social skills.¹¹

THE GENETIC ARCHITECTURE OF AUTISM: EVIDENCE FOR NUMEROUS, INDIVIDUALLY RARE GENETIC CHANGES

Data from sibling and twin studies provide strong evidence of genetic heritability greater than 90%.^{12, 13} In light of this, numerous studies have been conducted to identify autism-associated

loci and candidate genes. Chromosomal abnormalities have been observed in about three to five percent of autism cases, with abnormalities in the 15q11-13 locus accounting for about one percent of cases.^{13, 14} G-banded karyotype analysis has been a standard method for detecting autism-associated chromosomal abnormalities for about 35 years. This microscopic analysis of the 46 chromosomes can reveal large chromosomal rearrangements, deletions, and duplications as small as 3 Mb, although it is not uncommon for 5-10 Mb abnormalities to go undetected.¹⁵ Recently, submicroscopic cytogenetic abnormalities have been investigated via microarray-based comparative genome hybridization (array-CGH) studies.

Arguably, the most significant findings of array-CGH studies relate to copy number variants (CNVs): genomic regions larger than 1 kb harboring insertions, deletions, duplications, or more complex variations. Although CNVs are a common form of genetic variation, some—including those in the 15q11-13 locus—are observed more frequently or exclusively in autistic individuals.^{14, 16} The majority of autism-associated CNVs are rare, with the most common recurrent CNVs accounting for at most two percent of autism cases and the least common occurring within a single family.¹⁷ Many of these CNVs are *de novo*, while others are inherited. Notably, autism-associated CNVs do not appear to act in a Mendelian fashion, in contrast to most classic genomic disorders.

To date, a large number of individually rare genetic changes—including *MECP2* mutations and numerous CNVs—have been associated with autism. Because genetic testing with microarrays has entered the clinical lab and because there are novel high-throughput sequencing technologies under active research, these insights have important diagnostic relevance, which is well exemplified by the case below.

AUTISM AND THE 15q11-13 LOCUS: A CASE

CB is a 16-year-old girl whose first developmental concerns arose at four to five months of age. At that time, she was not visually fixing on faces. She did not smile responsively or laugh. She remained extremely quiet through her first year of life, with only minimal babbling. Language did not develop until approximately three years of age. Rather than go through a period of babbling, she exhibited echolalia. She was able to pronounce words clearly, but did not use them in an appropriate context.

CB engaged only in solitary play until she was approximately three years old, when she began to interact with her older sister. It was not much later that she began to interact with her parents. She did not exhibit joint attention until nine years of age. Even after she began interacting, her play was repetitive. She was very rigid in her behaviors and craved structure. CB had poor attention. She developed simple motor tics including throat clearing. She displayed features of sensory integration disorder; she did not allow anyone to touch her hands and there were certain textures of food that upset her greatly. Over time, CB displayed severe obsessive compulsive symptoms. She has never had any seizures.

The family history was limited since the patient was adopted, but her biological mother was known to have cognitive delays and social impairments. The patient has a half sister by the same mother who has a seizure disorder, cognitive delays, and social impairments.

The physical examination was notable for short stature and almond-shaped eyes. She was otherwise non-dysmorphic. The neurological examination was notable for pressured, perseverative speech involving a limited array of topics. She repetitively asked the same questions and dictated the topics of conversation. CB exhibited poor attention, motor tics, and obsessive compulsive behaviors. She insisted that the examiner use the same stethoscope that had been used during previous visits and that the order of the examination not deviate from previous visits. The remainder of the general and neurological examinations was normal.

To evaluate CB's developmental concerns, she underwent fluorescent in-situ

hybridization (FISH) of the 15q11-13 region. This revealed a duplication within 15q12.

The 15q11-13 locus is highly unstable and subject to genomic imprinting. Of 58 CNVs observed in this locus, 45 are associated with disorders, including Prader Willi Syndrome, Angelman Syndrome, autism, intellectual disability, and schizophrenia.¹⁸ A number of genes within this locus, such as *UBE3A* and members of the GABA receptor gene family, are expressed throughout the central nervous system and have been associated with autism or other neuropsychiatric disorders.¹⁹

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Variations at the 15q11-13 locus are observed in up to one percent of autistic individuals, and the majority of the numerous observed variations are maternally-inherited duplications.¹⁴ Many of CB's symptoms are in accordance with those described in other patients harboring 15q11-13 duplications, including developmental delay, echolalia, lack of major facial dysmorphisms, and short stature.²⁰ As expected, many of CB's symptoms overlap with ASD symptoms, including delayed development of language, abnormal social interaction, solitary and repetitive play, sensory integration differences, and strong desire for structure. Based on the family history, it is likely that both CB and her half-sister inherited the duplication from their mother.

Of note, a recent physician advisory (http://www.idic15.org/PhysicianAdvisory_Feb2009.pdf) warns that medications that target the GABA-A receptor—including benzodiazepines, phenobarbital, and ethanol derivatives—may increase the risk of sudden death in individuals with

15q11-13 duplications. Further research is necessary to definitively establish a link between these medications and sudden death in these individuals; however, the fact that the 15q11-13 locus includes several GABA receptor genes provides biological plausibility for this clinical concern.

In summary, CB now has a diagnosis of "autism associated with 15q11-13 duplication." This molecular subtype of autism provides a biological explanation, has relevance to recurrence risk, may have significant implications for treatment (e.g. caution in prescribing benzodiazepine, phenobarbital, or ethanol derivatives) and may help predict the clinical course.

DIAGNOSIS IN THE MULTIDISCIPLINARY CLINIC: THE ADVANTAGES OF GENETIC TESTING

Given the wide spectrum of autistic phenotypes, it is fitting that diagnosis and management of this complex disorder is becoming increasingly collaborative. It is common for patients to be seen by a number of professionals, including pediatricians, psychiatrists, neurologists, medical geneticists, and speech and behavioral therapists.

A crucial collaboration is that between clinicians and research geneticists. Although autism remains a behaviorally-defined disorder in DSM-IV-TR, approximately ten percent of ASD cases have known cytogenetic causes⁸ and more discoveries are certainly on the way, thanks to the advent of array-CGH technology and the rapid advancement of the field. Discovery of the genetic causes of specific forms of autism will allow these molecular subtypes to be added as descriptors of the behavioral diagnosis, much in the way that *MECP2* mutations in Rett's disorder have provided important biological explanations.

More widespread use of genetic testing for autism would enable earlier detection and, therefore, intervention in a sizable fraction of patients. The benefit of early diagnosis and intervention is highlighted by experiments involving animal models of the monogenic disorders Fragile X syndrome and Rett Disorder, in which early treatment was shown to delay or prevent the onset of autistic symptoms, as well as decrease their severity.¹³

Genetic testing has additional advantages. Firstly, identification of genetic causes of autism not only provides bio-

logical explanations for families but also may help to reduce the stigma around this disorder and prevent the development of false, damaging explanations for autism.²¹ Secondly, a specific genetic diagnosis may reveal information important to a patient's medical future, such as increased risk of sudden death for 15q11-13 duplication patients being treated with GABA-A agonists (http://www.idic15.org/PhysicianAdvisory_Feb2009.pdf) or increased susceptibility to cancer for individuals with *PTEN* mutations.²² Thirdly, given the high risk of autism recurrence among siblings, a specific genetic diagnosis in one individual may aid in the identification of autism or related neuropsychiatric disorders such as epilepsy, and/or associated susceptibilities to diseases such as cancer in family members.^{21, 23}

A recent consensus statement¹⁵ proposed that microarray analysis should replace G-banded karyotype analysis as the standard genetic test for autistic patients. This recommendation is based on the fact that microarrays have a resolution more than ten times that of karyotypes as well as a diagnostic yield about six times greater. Furthermore, the cost of microarray testing is lower than that of karyotype analysis followed by specific FISH testing.

As the cost of genetic testing decreases and clinical microarrays become more widely available, this technology will almost certainly become a standard aspect of autism diagnosis. As we continue to identify the genetic causes of the numerous subtypes of autism, more and more people on the spectrum can benefit from genetic testing, earlier diagnosis, and earlier intervention. Appreciation of the multidisciplinary nature of autism will enhance the rate of discovery of autism-associated loci and facilitate improved diagnosis and treatment of autistic patients, thus improving the lives of individuals and families affected by autism. While progress in autism genetics has made significant contributions to the clinic, much research remains to be done.

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