

Neurodevelopmental Disorders and Genetic Testing: Current Approaches and Future Advances

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Genetic testing for intellectual disability, global developmental delay and other neurodevelopmental disorders has advanced considerably in the last five to ten years and can be an important diagnostic tool for clinicians. This article provides a clinical and ethical framework for understanding these advances, future directions and the current limitations of these approaches.

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Children with significant developmental cognitive and behavioral disabilities (often grouped under diagnoses of global developmental delay [GDD], intellectual disability [ID], and autism spectrum disorder [ASD]), require significant investments from clinicians, educators, and family members to help them reach their full potential. The optimal approach to helping these individuals often begins with a precise diagnosis. This enables targeted treatment plans, provides anticipatory guidance, and gives parents actionable information for family planning. Our previously published guideline and recently updated evidence report outlined a broad-based diagnostic algorithm that focused on genetic and biochemical testing.^{1,2} However, the rapid and accelerating pace of advances in genetic testing (particularly chromosomal microarray analysis [CMA], whole exome sequencing [WES], and soon whole genome sequencing [WGS]) warrants expanding upon that discussion to consider how next generation testing modalities can be rationally and ethically introduced into clinical practice.

In this review, we define the patient populations that comprise neurodevelopmental disorders (NDDs), review current and newly evolving diagnostic tools for

NDDs, and provide our recommendations for utilizing these tests in the current environment and going forward. We are optimistic that framing the discussion in this manner will allow clinicians to chart a path toward better care for their patients with NDDs.

NDDs

GDD and ID are nonsynonymous NDD subtypes.¹ GDD applies to children younger than 5 years and is defined as significant delay (2 standard deviations below the mean) in 2 or more developmental domains (ie, motor [gross, fine], communication/language, social, cognitive, and activities of daily living).³ In contrast, ID is a disorder of cognition that can be made more confidently after the child is 7 years or older and does not necessarily involve motor deficits.^{4–6} In addition, children with GDD or ID may demonstrate 1 or more autistic traits that may or may not meet the diagnostic threshold for a diagnosis of ASD. There is significant overlap between ID and the broad autism phenotype.^{7,8} Studies suggest that up to 70% of children with a clear diagnosis of autism will have a diagnosis of ID.⁹ This has important implications for genetic testing. Etiologic

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testing and complementary treatment plans rely on a comprehensive clinical assessment, ideally involving an interdisciplinary team of pediatric health care professionals.

Genetic Tools for Diagnosis and Discovery

ID affects up to 2% of the population.² Current genetic platforms identify causative mutations in up to 25% of cases, and new tools in development offer the promise of even higher diagnostic yield. Several currently available genome-wide tools (CMA and more recently WES) can identify causal genetic variants associated with ID, GDD, and ASD.¹⁰⁻¹⁵ Each technology has its own technical, financial, and interpretative limitations. CMA has in a very short time helped to delineate new phenotypes in children with ID, autism, and neuropsychiatric disorders.¹⁶ Recent reports on WES suggest that this too will add considerably to our understanding of the genetics of NDD.^{14,17}

CMA in the Diagnosis of Children with ID/GDD

Recent evidence indicates that genomic structural variation plays a significant role in susceptibility to disorders associated with ID and ASD. A copy number change reflects a deletion or duplication of a genomic region compared to a normal reference genome. These copy number variations can range in size from several kilobases to several megabases or an entire chromosome. The introduction of genome-wide array platforms has vastly improved the ability to identify chromosomal copy number changes beyond karyotype and fluorescence in situ hybridization.

CMA has the highest diagnostic yield of any single clinically available test for children with ID, ASD, and/or congenital anomalies.¹⁸ The yield for clinically significant copy number variations (CNVs) can be as high as 15 to 20%.¹⁹ These results may impact prognosis, identify and direct management of medical comorbidities, and inform recurrence risk counseling. Reflecting this utility, the 2010 American College of Medical Genetics practice guidelines recommended chromosome microarray as a first-tier test for children with multiple anomalies not specific to a well-delineated genetic syndrome, or for cases of nonsyndromic ID or ASD.²⁰ Additionally, several studies have shown that CMA often informs medical management (ie, cancer risk and need for surveillance in advance of symptoms).²⁰ Several new microdeletions have been identified in patients displaying a characteristic and highly reproducible phenotype (eg, 17q21.31 deletion). However, other microdeletions and duplications have been identified in

association with multiple phenotypes of varying severity.¹⁰ Many of these more clinically variable but recurrent CNVs were only discovered through the use of these new genetic tests. Currently, the 2 CMA techniques in most frequent use are comparative genomic hybridization and single nucleotide polymorphism (SNP) arrays. SNP arrays have the advantage of also detecting absence or loss of heterozygosity. Regions of homozygosity due to uniparental disomy or consanguinity can also be identified and screened for pathogenicity.²¹ Not only do these array platforms have great diagnostic potential, but concerted efforts from many laboratories have begun to delineate the natural history of newly recognized disorders (<http://sfari.org/sfari-initiatives/simons-vip>).²² Furthermore, as CMA is used with greater frequency and there are data on the detection of CNVs in large control cohorts, it is increasingly possible to assess the pathogenicity of individual rare CNVs.^{16,22,23} This knowledge is of critical importance, as many laboratories are now testing for CNVs prenatally,²⁴ including testing fetal cells for preimplantation genetic diagnosis.²⁵⁻²⁸

Despite the advantages of CMA, there are some notable limitations. Because only unbalanced copy number changes are detected, arrays cannot identify balanced inversions/insertions or reciprocal translocations. Likewise, because of the overall resolution, arrays also will miss low-level mosaicism (typically <20%), and point mutations or small insertions or deletions in single genes. Thoughtful interpretation of the microarray results has been previously published,^{18,29} but an additional discussion of “variants of unknown significance” is warranted. These are variants that are rarely seen in the general population or are unique to a family (frequency < 0.001) and are not a priori known to be associated with disease. It can be difficult to determine whether such rare CNVs are pathogenic or benign, and this determination will only occur with additional data collected from large populations. The difficulty of this determination is supported by the observation that up to 12% of euchromatin varies between individuals with no apparent phenotypic effects.³⁰ However, data from meta-analyses and from large cohort tests suggest that large (>1Mb), gene-rich deletions are more likely to be found in patients with GDD/ID than in control populations.^{18,22} Moreover, CNVs that are de novo or that are large and inherited from an affected parent are also more likely to be pathogenic. For an increasing number of CNVs, there is now enough accumulated data to begin to estimate their pathogenicity.³¹ Clinical information about rare CNVs is available through frequently updated public sources such as the International Standards for Cytogenomic Arrays Consortium. Established in 2007, this worldwide organization

involves >150 clinical laboratories and has goals that include standardization of genotype and phenotype data and evidence-based data interpretation by expert clinicians. Two public databases, the Database of Genomic Structural Variation (dbVar), and ClinVar are maintained by the National Center for Biotechnology Information and provide complementary data on genomic variation, with dbVar providing data on all genomic variants without the need to contribute significant phenotypic data and ClinVar focused on genomic variants of clinical significance. These databases, coupled with data collected from parental testing and the clinical expertise of physicians and cytogeneticists, are part of the essential framework for interpreting CNVs on a patient-by-patient basis.

Next Generation Sequencing

Current genetic testing using Sanger sequencing allows for single and multigene sequencing tests for a number of rare disorders. Panels of tests are currently available for a number of phenotypes, including ASD, epilepsies, mitochondrial disorders, ataxias, disorders of glycosylation, and X-linked ID. These panels provide detailed sequencing of the specific genes that are highly associated with a phenotype. In comparison to WES (see below), the individual genes are sequenced more completely and reliably. When gene panels were performed using Sanger sequencing, the process was quite expensive, leading to fewer genes being included. Laboratories using next generation sequencing (NGS) for gene panels are able to efficiently and cost-effectively screen much larger numbers of genes.³²

WES

More recently, WES has also become clinically available. *Exome* refers to all of the exons in the human genome, which constitute about 1% of its total length, approximately 30Mb. Current WES platforms include untranslated regions and other highly conserved noncoding DNA, such that up to 70Mb are sequenced. Mutations within the exome account for about 85% of all disease-causing mutations so far identified (although this result does suffer from ascertainment bias).³³ WES has been reported recently to be an effective research method for identifying the genetic cause of an increasing number of rare Mendelian disorders. This means of investigation is a rapidly accelerating area for discovery.^{34–38} The National Human Genome Research Institute has recently established 3 centers tasked with advancing this process (<http://www.mendelian.org>), and >100 disorders have been targeted for WES study, many of which are associated with ID. A similar approach, when used in a cohort

of consanguineous families affected with ASD, identified a number of novel candidate ASD genes.³⁹

In addition to inherited Mendelian disorders, there also is accumulating evidence that *de novo* point mutations (as well as insertions and deletions too small to be seen by current microarray technology) are an important cause of genetic burden in both ID and ASD. Vissers and colleagues⁴⁰ were the first group to publish on this hypothesis using exome sequencing, examining an affected child with both parents serving as control samples to search for *de novo* genetic events. They conducted this family-based analysis by exome sequencing 10 individuals with unexplained ID (and their unaffected parents), identifying 9 unique *de novo* mutations in 9 genes, 6 of which were likely to be pathogenic, based upon gene function and the type of mutation. A similar approach has now been undertaken in large ASD cohorts and in 2 cohorts with severe ID. The ASD cohorts included families with 1 affected and 1 unaffected child.^{13,15,41,42} This approach allowed investigators not only to identify *de novo* mutations, but also to compare the rate of mutations in the exomes of healthy and affected siblings (no change in overall *de novo* mutation rate). This design also permitted an analysis by mutation type and by gene grouping, enhancing the ability of the investigators to address mutation pathogenicity. Methodologically, a number of different informatics platforms are first fine-tuned to distinguish between a biological variant and a false positive and then are used to distinguish between a polymorphism and a pathogenic mutation. Similar to CNV analysis, any potentially pathogenic finding is confirmed by testing in another platform. For NGS, this is done through Sanger sequencing. Using this approach, these laboratories have shown that highly disruptive (nonsense and splice-site) *de novo* mutations were enriched in ASD patients versus their unaffected siblings and that the mutations were more likely to be found in genes expressed in the brain or in genes involved in pathways previously implicated in ASD. Many of these novel genes were only found mutated in 2 or 3 cases (or just 1). To provide additional evidence implicating these genes as causative for ASD, investigators have conducted less expensive targeted follow-up sequencing, using a novel platform for sample multiplexing, molecular inversion probes. This approach identified recurrent mutations in many candidate autism genes, including *KATNAL2*, *CHD8*, *DYRK1A*, *TBR1*, and *TBLIXR1*, as well as in genes previously implicated in ASD, such as *SCN2A*, *SHANK2*, *NTNG1*, *GRIN2B*, *LAMC3*, and *PTEN*. *De novo* mutations in this group of ASD genes may account for up to 3% of children with ASD, and further sequencing will likely identify many other genes with recurrent

severe de novo mutations, providing strong evidence that they are important in pathogenesis.

For ID, 2 recent papers address pathogenicity through an analogous approach examining family trios for de novo mutations.^{14,17} These studies also found that highly disruptive mutations were more prevalent in patients with ID than in controls and found recurrent mutations in a number of genes, both novel (*DYNCH1*, *GATAD2B*, and *CTNNA1*) and previously identified (eg, *SCN2A*, *STXBPI*, and *SYNGAP1*). Many of these patients with profound ID also had ASD, epilepsy, and malformations of cortical development. This overlap between mechanisms for these different but often linked conditions may have important implications for treatment.

The repeated discovery of mutations in the same set of genes implicates the mutations as pathogenic and suggests that, as is happening with CNVs, testing larger numbers of patients will provide further discovery and validation and allow genotype–phenotype correlations to be made. However, as for CNVs, many single nucleotide variants (SNVs) that are being detected at this early stage are of uncertain significance, although the de novo highly disruptive variants are very likely to be pathogenic. Thus, for both CNVs and SNVs, de novo mutations appear to be associated with many NDD phenotypes. Regarding the overall genetic contributions to NDD, some studies point to a combination of de novo mutations and an excess of inherited rare pathogenic mutations.⁴³ What percentage of the overall genetic burden comes from de novo and inherited mutations is not yet clear.⁴⁴ However, 2 recent studies of ASD suggest that each commonly inherited SNP contributes only a small amount to the risk of autism, but that the combined influence from inheriting many of these SNPs may be greater.^{45,46} As many patients with autism also have ID, it is likely that low-penetrance common variants have a similar effect on the risk of ID.⁴⁷ How clinicians can best incorporate analysis of these low-penetrant variants into diagnostics is still unclear. Nevertheless, many clinical laboratories have started to offer WES to search for highly penetrant de novo mutations (and rare recessive Mendelian diseases) in patients with ID/GDD.⁴⁸ This effort, like the effort for CNVs, will accelerate the pace of discovery of syndromes and critical phenotype–genotype correlations. Moreover, like CNVs, a proper clearinghouse of genetic variants accompanied by a rich phenotypic database will be needed to make sense of the vast array of these findings.

Genome Testing: Clinical and Ethical Implications

A more complete understanding of the genetic contributions to ID/GDD is still developing, but it is clear that

work done over the past 2 decades has already shed considerable light on the subject and that clinical laboratories are providing rich data sets that are fueling this progress. CNV analysis is already a regularly used and reliable tool for the evaluation of patients with ID/GDD, and WES is likely to find similar acceptance.

Obtaining a genetic diagnosis (whether targeted or genome-wide CNV or WES based) can help in the relief of parental anxiety and guilt, improve access to support and research networks, avoid further diagnostic testing that may be costly or invasive, improve understanding of treatment and prognosis, provide better anticipation and management of associated medical and behavioral comorbidities, facilitate more accurate counseling regarding recurrence risk, and enhance opportunities to prevent recurrence through screening for carriers and prenatal testing.^{2,20} Moreover, determining whether a genetic abnormality is de novo or inherited provides essential and actionable information for the family.

Many metabolic disorders are inherited in an autosomal-recessive manner and are hence more likely to be identified through WES than through microarray testing.^{37,49} Such a diagnosis would have the most direct and immediate influence on patient management, given the availability of specific dietary treatments and enzyme replacement therapies that can, for certain metabolic disorders, improve clinical outcomes.^{50–54} There are also increasing examples of genetic diagnoses positively influencing pharmacological choice or disease surveillance. Finding an *SCN1A* mutation⁵⁵ or a *GLUT1* defect⁵⁶ would lead to highly specific changes in interventions that enhance seizure management and developmental outcomes. Finding a mutation in a known tumor suppressor gene, such as *PTEN*, can reveal a need for cancer surveillance.^{57,58} Several papers have shown how positive genetic test results in children with ID guides follow-up testing and referral recommendations.^{59,60}

Our understanding of how to best manage children with genetic disorders causing ID is rapidly expanding, in part because of the widespread availability of testing. Fragile X syndrome (FXS), due to a trinucleotide expansion adjacent to the *FMRI* gene, is the most commonly inherited cause of ID. The genetic etiology for FXS was identified in 1992.⁶¹ Now, only 20 years later, there are dozens of clinical trials exploring the efficacy of novel and repurposed compounds to treat the behavioral difficulties encountered by these patients.^{62,63} This progress was in part possible because of the widespread screening for this genetic etiology of ID. Moreover, some of the more recently identified causes of ID and ASD have overlap with FMR signaling.^{42,64} It would be a reasonable hypothesis to suggest that medicines that treat FXS

may also help patients with mutations in overlapping biological pathways (as has been recently demonstrated in animal models^{65,66}). Thus, obtaining a proper genetic diagnosis has the possibility of directing patients toward targeted treatments. Moreover, the complexity of different genetic diagnoses, even if they may converge on common signaling pathways, warrants this broad-based genome-wide testing approach.⁶⁷

Not unexpectedly, as the scope and yield of these newer genetic tests have expanded, so initially have the costs. This has been particularly evident for whole genome analyses. However, multiple recent studies have shown the clinical utility and cost-effectiveness of CMA over karyotyping or other stepwise approaches and have documented a significant drop in costs since the first generation of tests.^{59,68} As the cost of WES has dropped substantially in the past few years, and the ability to analyze the full data set has increased, we anticipate that this approach will rapidly become an equally valuable companion to microarray testing in children with ID/GDD.⁶⁹

In addition to these advances in understanding the genetic basis of ID/GDD, in parallel there have been similar advances in other broad-based platforms, such as metabolomics and proteomics, that hold similar promise for their ability to be used for disease discovery and clinical diagnosis.⁷⁰

Ethical concerns exist regarding both CNV testing and WES (and ultimately WGS). With CNV testing for postnatal disorders such as ID and GDD, concerns have focused on whether consent in such a setting can truly be informed.⁷¹ This largely relates to the possibility of discovering genetic risks for outcomes unrelated to the developmental disorder that initially prompted testing.⁷² Clinicians cannot possibly convey sufficient information about all findings to ensure that parental decision making is truly informed. This has led to consideration of a “generic” consent that allows parents the option of choosing not to be informed about test outcomes unrelated to ID and GDD.⁷³ With respect to genomic sequencing, consideration of ethical challenges has focused primarily on managing the huge amount of resulting genetic information.⁷⁴ Both WES and WGS are likely to bring a plethora of results that are unrelated to the child’s developmental disability.⁷⁵ Information-processing systems need be in place to correctly categorize the variants that are found. Such systems will need to first filter out artifacts, variants known to be benign, and variants predicted to be benign due to location.⁷⁶ Even with such filtering, novel variants of potential or uncertain pathogenicity will be found. In 1 recent case, WES revealed 79,525 genomic variants in a pair of monozygotic twins, with 32 novel variants in 32 genes ultimately remaining after rigorous

filtering.⁷⁶ At present, guidelines from the National Human Genome Research Institute Institutional Review Board mandate communication of incidental genetic information if: (1) the genetic change is of “urgent” clinical significance; (2) early diagnosis would alter reproductive and medical decisions, making it clearly beneficial to impart the information; (3) knowledge of the potential genetic disorder outweighs the risks associated with anticipatory anxiety and subsequent medical testing; or (4) the variant is inherited in a recessive fashion, the frequency of the disease is >1 in 40,000, the disorder results in significant morbidity, and early diagnosis and treatment of the disorder confers a clear benefit.⁷⁶ Implementing such guidelines, considering current workforce availability, is impractical and unlikely, suggesting the need for careful study of how the health care system can meet these challenges so that a balanced approach is feasible.

There is no single approach to diagnostic testing that will fit the needs of every child and family. Clinical judgment remains paramount in deciding whether genetic, imaging, or metabolic testing is warranted, in what sequence, and to what degree. It is not our recommendation that all tests be done on all patients with developmental disorders. However, for patients whose severe ID or autism is unexplained, the steady technical improvements and declining costs of genome-wide testing platforms, and the compelling evidence that many cases of ID/GDD can be explained by highly penetrant genetic causes makes the case for physician-guided implementation of these tests. Regardless of how clearly a test result will influence a child’s clinical management in the short term, or advance the progress of science, the great value that most families put on a definitive diagnosis alone deserves consideration.^{77,78}

Potential Conflicts of Interest

E.H.S.: grants/grants pending, NIH, Simons Foundation, CURE; stock/stock options, Ingenuity Systems. D.J.M.: travel expenses, American Academy of Neurology Guideline Development Subcommittee, Institute of Medicine. A.L.G.: consultancy, GeneDx; grants/grants pending, National Urea Cycle Foundation, Propionic Acidemia Foundation, NIH; paid educational presentations, Shire HGT; guest editor, *American Journal of Medical Genetics*.

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