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Updates in the Genetic Evaluation of the Child with Global Developmental Delay or Intellectual Disability

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Global developmental delay (GDD) and intellectual disability (ID) occur in up to 3% of the general population and are even more commonly encountered in the setting of the pediatric neurology clinic. New advances in technology and in the understanding of genetic disorders have led to changes in the diagnostic approach to a child with unexplained GDD or ID. Chromosomal microarray has become a first-line test for evaluation of patients in this population and has both significantly increased diagnostic yield and introduced new challenges in the interpretation of copy number variants of uncertain significance. The G-banded karyotype is now frequently utilized as an adjunct to the microarray rather than as a first-line test in individuals with GDD or ID. Fragile X DNA testing continues to be recommended in the initial evaluation of the child with GDD or ID. The presence or absence of certain cardinal features (such as microcephaly or macrocephaly, seizures, autism, abnormal neurologic examination, and facial dysmorphism) can be utilized to direct single-gene molecular testing. The availability of next-generation and massively parallel sequencing technologies has enabled the use of genetic testing panels, in which dozens of genes associated with GDD or ID may be rapidly analyzed. Most recently, the clinical availability of whole-genome and whole-exome sequencing has opened new possibilities for the evaluation of individuals with GDD or ID who have previously eluded a genetic diagnosis. Consultation with a medical geneticist is recommended when progressing beyond first-tier analyses to most efficiently prioritize testing.
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Introduction

Global developmental delay (GDD) and intellectual disability (ID) affect up to 3% of the general population and are a common reason for referral to pediatric neurologists.[1,2] GDD is typically used to describe children under the age of 6 years who have significant delay ($\geq$ 2 standard deviation below age-matched peers) in 2 or more areas.[3] Many of these children would later be diagnosed with ID, which is most commonly defined as having an IQ$\leq$-75 in addition to impairment in adaptive functioning.[4]

New technology and recent advances in the understanding of genetic disorders have allowed the development of genetic tests with greater sensitivity in elucidating an etiologic diagnosis for GDD or ID. Patients and families may benefit from establishment of a genetic diagnosis for multiple reasons. First, a specific diagnosis allows for a better understanding of the etiology and prognosis of the disorder. It may inform treatment decisions for associated symptoms (such as seizures, behavioral difficulties, and others), often allows specific anticipatory guidance recommendations, and permits patients and families to have access to appropriate support groups, research studies, and educational services. Accurate recurrence-risk information can only be provided to family members after a precise genetic diagnosis is elucidated. If a causative genetic alteration is detected, preimplantation and prenatal diagnosis may then be available to at-risk relatives contemplating a pregnancy. Additionally, arriving at a definite genetic diagnosis can limit further, often costly, diagnostic testing.

When considering a genetic testing strategy, important factors include the likely diagnostic yield of the test. The estimated diagnostic yield of various testing modalities (eg, karyotype, chromosomal microarray [CMA]) among individuals with GDD or ID has been investigated in multiple prior studies.[2,5-7] However, the likelihood of

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obtaining a diagnostic result on a specific genetic test is dependent on many other factors, including the patient's specific clinical history, ethnicity, and family histories; presence or absence of characteristic features on examination; and the specific test methodology (which may influence detection rate). Other considerations include the availability, cost, and invasiveness (eg, peripheral blood sample vs muscle biopsy) of the test. This review focuses upon the current strategies in diagnostic genetic testing for children with GDD or ID, including cytogenetic and molecular genetic analyses. Evaluation for primary biochemical disorders is not specifically addressed in this review; however, inborn errors of metabolism should be strongly considered in individuals with specific clinical features (episodic decompensation, regression in developmental milestones, and abnormal myelination on brain magnetic resonance imaging), positive family history, and specific physical findings (coarse facial features and hepatosplenomegaly).[2]

First-Line Genetic Testing

If a child has an identifiable or probable genetic syndrome for which genetic testing is available, this analysis should be performed first. For example, on the one hand, CMA would not be an appropriate initial test for an infant with features consistent with Down syndrome, for which the recommended confirmatory test is a routine karyotype. If, on the other hand, a detailed clinical history, physical examination, and family history does not suggest a specific disorder, the following analyses should be considered first line for a diagnostic genetic evaluation of an individual with GDD or ID (Fig 1).

CMA

CMA is increasingly becoming the most commonly ordered initial genetic diagnostic test in patients with unexplained GDD or ID. A 2010 consensus statement, based on a meta-analysis of 33 studies by the International Standard Cytogenetic Array (ISCA) Consortium, indicated that CMA should be the first-line diagnostic test for individuals with GDD or ID, autism spectrum disorders, or multiple congenital anomalies.[8] CMA permits genome-wide detection of copy number variants (CNVs) at a significantly higher resolution than G-banded karyotyping. Currently available CMA platforms allow the detection of CNVs with a minimum resolution of ~100 kb throughout the genome, compared with a lower limit of detection of ~3-5 Mb on G-banded karyotype, representing a 30-fold to 50-fold increase in resolution.[9] Therefore, CMA allows improved detection of pathogenic microdeletions and microduplications, in addition to detection of cryptic genomic imbalances associated with apparently balanced chromosome rearrangements and more precise characterization of unbalanced chromosome rearrangements detected on G-banded karyotype.[10] The 2 main types of CMA currently used are the comparative genomic hybridization array and the oligonucleotide array (including the single nucleotide polymorphism [SNP]–based array). The added advantage of SNP array is its ability to detect long stretches of homozygosity, which might represent uniparental disomy

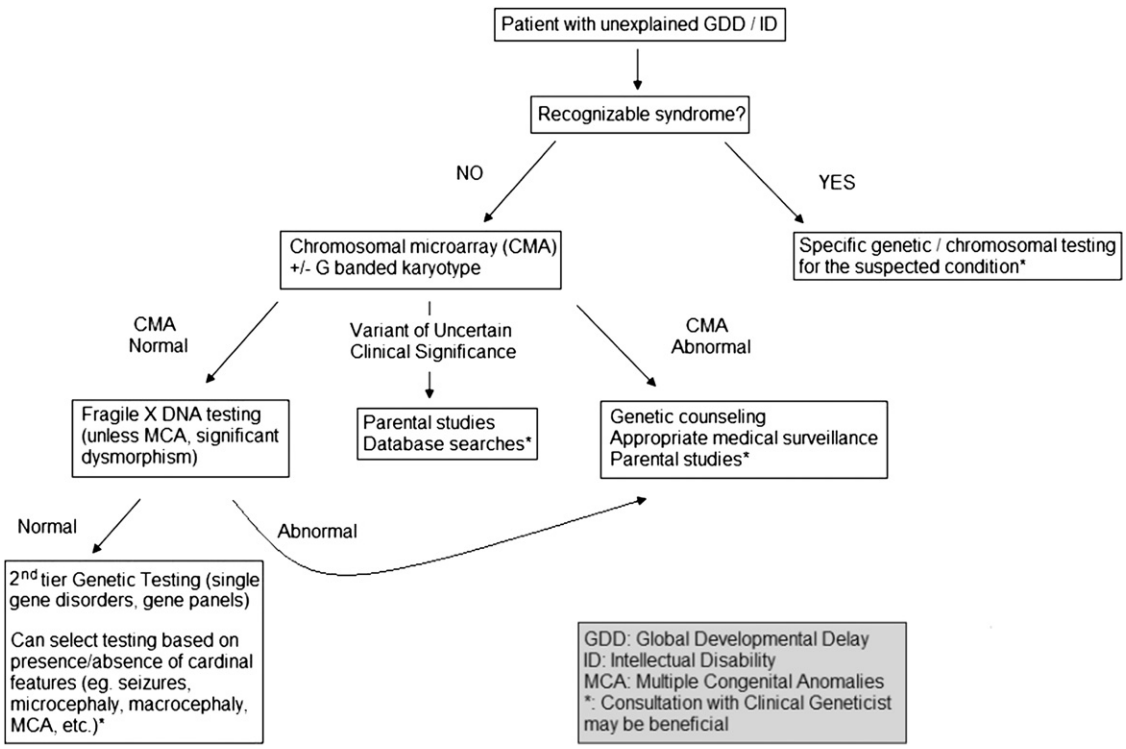

Figure 1 Suggested initial genetic testing approach for the child with GDD or ID.

(UPD) or unsuspected consanguinity, and its increased sensitivity to chromosomal mosaicism compared with other types of CMA.[8,11] Most clinical laboratories recommend confirmation of chromosomal deletions or duplication detected by CMA with another methodology, typically either fluorescence in situ hybridization (FISH) or multiplex ligation-dependent probe amplification analysis of the specific genomic region involved. Subtelomeric FISH, previously used to detect deletions and translocations involving the distal portion of the chromosomes in individuals with GDD or ID, has been replaced by CMA, which detects CNVs in these regions as well as throughout the rest of the genome.

The diagnostic yield of CMA has been clearly established to be higher than the G-banded karyotype in individuals with GDD or ID, autism, and multiple congenital anomalies. Traditional chromosome analysis yields a diagnosis in approximately 5% of individuals with ID, whereas CMA uncovers an underlying etiology in about 10%-20%.[8,11] The yield is even higher in individuals with more severe ID, multiple congenital anomalies, and dysmorphic features.[12] Table 1 contains a selected list of well-characterized microdeletion and microduplication syndromes causing GDD or ID.

CMA also frequently detects CNVs of uncertain clinical significance, which can present a diagnostic and counseling

Table 1 Selected Chromosome Microdeletion and Microduplication Syndromes

Location	Size/Dup or Del	Neurocognitive Features	Other Features	Unaffected Parent Described?	Selected References
1q21.1	~1.35 Mb/ Del	Mild-moderate developmental delay (>75%), ID (25%-50%), microcephaly (50%-75%), autism (<10%)	Short stature, failure to thrive, ophthalmologic abnormalities, mild dysmorphic features, and an increased prevalence of cardiac and renal anomalies	Yes	13,14
1p36	Variable/ Del	Developmental delay or ID (severe-profound in 90%), hypotonia (95%), seizures, structural brain anomalies, microbrachycephaly (65%), and large and late-closing anterior fontanel	Dysmorphic facial features, brachycamptodactyly, short feet, congenital heart defects, ophthalmologic abnormalities, sensorineural hearing loss, and genitourinary malformations	No	15-17
15q13.3	~2.0 Mb/ Del	Highly variable. Developmental delay, ID (usually mild) in up to 50%, autistic features (up to 10%), attention difficulties, mood disorder, impulsive behavior, and schizophrenia	No recognizable pattern of dysmorphic features and possible increased prevalence of cardiac defects	Yes	18-20
15q24	~1.1 Mb/ Del	Developmental delay or ID in all reported individuals (mild-severe) and abnormal brain MRI (25%-50%)	Dysmorphic facial features, digital anomalies (~60%), genital anomalies (30%), and ocular anomalies	No	21,22
16p11.2	~550 Kb/ Del	Developmental delay (especially speaking ability) in majority, mild ID, autism spectrum disorder, and psychiatric disorder including schizophrenia	No recognizable pattern of dysmorphic features. Obesity and possible increased prevalence of cardiac defects	Yes	23,24
16p11.2	~550 Kb/ Dup	Highly variable. Developmental delay, autism spectrum disorder, and psychiatric illness	No specific pattern of other features	Yes	23,24
17q21.31	550-650 Kb/ Del	Developmental delay, ID (>75%), childhood hypotonia, friendly disposition, and seizures (50%-75%)	Distinctive facial features, joint hypermobility, cardiac and renal anomalies, and normal growth	No	25,26
22q11.2	1.5 or 3 Mb/ Dup	Highly variable. Developmental delay (usually mild), ID (mild), and hypotonia.	Growth restriction, and no specific pattern of dysmorphic features.	Yes	27

Del, deletion; Dup, duplication; ID, Intellectual disability.

challenge to the provider and the patient's family. Information about CNV locations, gene content, and clinical correlations is available in several public databases and online tools, including the DECIPHER database, University of California, Santa Cruz Genome Browser, and the Database of Genomic Variants. The ISCA Consortium has published criteria to assist in the classification of CNVs of uncertain significance.[8] CNVs are more likely to be pathogenic when they are larger in size, overlap with known microdeletion or microduplication syndromes, are gene rich, and contain genes associated with known phenotypes. Additionally, targeted analysis of both parents is usually recommended to determine whether the CNV has been inherited or has occurred as a de novo event. Inheritance of a CNV from an unaffected parent may suggest that it is a benign variant; however, this must be interpreted with caution. It is now known that many microduplication and microdeletion GDD or ID syndromes have widely variable expressivity, even within members of the same family, and may cause very mild or no apparent cognitive delays in some individuals. Furthermore, it has been proposed that some CNVs may cause GDD or ID via a "2-hit" mechanism, whereby a chromosome microdeletion or microduplication acts as a significant risk factor for ID, and a second insult (co-inheritance of another CNV, an alteration in a single gene, or an environmental event) is needed to cause a clinically significant developmental phenotype.[28]

Traditional G-banded Karyotype and FISH Analysis

Prior to 2010, the G-banded karyotype was a standard, first-tier test for the evaluation of the child with unexplained GDD or ID, especially if there were concomitant dysmorphic features or congenital anomalies. Currently, controversy exists about whether a standard karyotype should be performed concurrently with CMA; only after a normal CMA result is obtained; or not at all, except in certain clinical circumstances. Given the superior resolution and diagnostic yield of CMA, the ISCA Consortium has recommended performing traditional cytogenetic analysis only if the patient has a recognizable chromosomal syndrome that would be detectable on karyotype (eg, Turner syndrome), a family history of chromosome rearrangements, or history of multiple reproductive losses or infertility.[8,11] For patients with recognizable chromosome microdeletion or microduplication syndromes (eg, Williams syndrome, velocardiofacial or DiGeorge syndrome), FISH analysis for the specific syndrome can be used as an initial diagnostic test before CMA. However, because CMA is superior to FISH in detecting very small duplications or deletions, CMA should still be utilized if an individual with a suspected chromosome deletion or duplication syndrome showed a negative result in FISH analysis. The G-banded karyotype does have several advantages over CMA, including its ability to identify balanced chromosome rearrangements and low-level mosaicism that might be missed by certain types of CMA. G-banded karyotyping of a standard 20 cells can detect mosaicism to about a 14% level.[29] However, current SNP-based CMA may be able to detect chromosomal mosaicism as low as 5%.[30] The 2010 ISCA Consortium meta-analysis of 33 studies estimated that balanced translocations are identified in ~0.3% of individuals with ID who were tested with traditional chromosome analysis.[8] Some patients with an abnormal phenotype and an apparently balanced chromosome rearrangement, found on karyotype, may in fact have a submicroscopic gain or loss of chromosomal material, which may be detectable on CMA. The presence of a truly balanced rearrangement may still potentially be an explanative of GDD or ID (especially if the rearrangement has occurred de novo or is present in an affected parent) if the break points of the rearrangement interrupt a gene leading to an absent or abnormal protein product (through gene truncation or creation of a fusion gene). These would be undetected if no karyotype is performed.

Fragile X DNA Analysis

Analysis of the *FMR-1* gene is still considered a first-tier test for the diagnostic evaluation of an individual with unexplained GDD or ID and is appropriate testing for both males and females with mild to moderate ID, especially if there are concomitant autistic features or a family history supportive of X-linked inheritance. CGG trinucleotide expansions (>200 repeats) within the *FMR-1* gene with concomitant aberrant methylation are causative of the fragile X syndrome. The fragile X syndrome is clinically characterized by GDD or ID (typically moderate in males and mild in females), behavioral abnormalities (frequently, autism spectrum disorder), characteristic features (especially in males; large head, long face, and prominent ears), connective tissue changes (joint laxity and mild aortic dilation), and macro-orchidism. When 55-200 repeats are present (premutation range), there is an associated risk to develop late-onset cerebellar ataxia and intention tremor (fragile X-associated tremor or ataxia syndrome), and females are at risk for premature ovarian failure. Previous studies have shown that *FMR-1* analysis has a diagnostic yield of ~2% in males and females with mild ID.[8] Clinical features that, when present, would decrease the likelihood of a diagnosis of fragile X syndrome include microcephaly, multiple congenital anomalies, or profound ID. Unless a patient's family history and clinical features are strongly suggestive of fragile X syndrome (in which case *FMR-1* analysis should be performed before any other genetic tests), CMA is typically performed first in the evaluation of the child with GDD or ID, as the estimated diagnostic yield is higher. *FMR-1* testing can be performed after a normal CMA, or concurrently.

Second-Tier Testing

Common Single-Gene Syndromes in Children With GDD or ID

Overview

In patients with normal CMA, G-banded karyotype, and *FMR-1* testing, consideration can be given to further evaluation with molecular analysis for selected single-gene causes for GDD or ID. Genetic causes for GDD or ID may roughly be divided into 2 categories: syndromic (in which there are additional medical, neurologic, or dysmorphic features) and nonsyndromic. The presence or absence of certain major clinical features can help guide decisions about the appropriateness of additional molecular testing. Such features include microcephaly (defined as occipitofrontal circumference [OFC] < third centile; congenital or acquired), macrocephaly (OFC > 97th centile), presence of autistic features, seizures, presence or absence of an abnormal neurologic examination (eg, hypotonia and tremor), structural brain abnormalities, presence or absence of congenital anomalies or dysmorphic features or both, and concomitant medical issues (eg, vision or hearing impairment, failure to thrive, and frequent infections) (Table 2). The severity of GDD or ID may also be useful in determining the diagnostic yield of a specific test. It is important to note that most genetic syndromes represent a spectrum and that not all affected individuals may present with "classic" features. For this reason, and to assist in determining the most appropriate, cost-effective testing strategy, consultation with a clinical geneticist should be strongly considered when progressing beyond first-tier testing.

Rett Syndrome or *MECP2*-Related Disorders

Mutations in the *MECP2* gene, located on the X chromosome, are associated with a wide range of neurologic phenotypes in males and females, including the so-called classic Rett syndrome, variant Rett syndrome, and ID in girls, and neonatal encephalopathy and ID in boys.[31,32] Classic Rett syndrome is characterized by a 6- to 18-month period of normal development followed by neurodevelopmental regression with loss of purposeful hand movement and deceleration of head growth. Seizures, ataxia, tremors, and breath-holding spells are the other common features. Variant Rett syndrome may be more or less severe than classic Rett syndrome. Duplications of the *MECP2* gene are causative of infantile hypotonia and ID, often with recurrent respiratory infections and seizures. Studies have shown that *MECP2* mutations are found in ~1.5% of girls with moderate to severe GDD or ID and in 0.2%-0.4% of boys with GDD or ID.[8] Molecular testing should include sequencing of all 4 exons of *MECP2* as well as gene duplication or deletion analysis.

Angelman Syndrome (AS)

AS occurs with equal frequency in males and females and is characterized by severe ID with near-absence of speech, gait ataxia, a distinctive behavioral phenotype with episodes of spontaneous laughter, and frequently, acquired microcephaly and seizures.[33] Girls with AS may occasionally be clinically misdiagnosed as having Rett syndrome; however, neurodevelopmental regression and loss of purposeful hand use are not characteristic of AS. AS is caused by decreased or abnormal function of the maternally inherited *UBE3A* allele on chromosome 15q11-q13. AS may arise from several different genetic mechanisms, including a 5- to 7-Mb deletion of this region on the maternally inherited chromosome (~70% of patients), paternal UPD (~7% of patients), mutation in the maternally derived *UBE3A* allele (~11%), and imprinting center defects (~3%).[34] Approximately 10% of individuals with a clinical diagnosis of AS do not have an identifiable genetic abnormality using current testing techniques. The preferred molecular testing method for evaluating an individual with a suspected diagnosis of AS is DNA methylation analysis, which would detect AS due to a deletion, UPD, or imprinting center defect, and has an estimated detection rate of 80%. If DNA methylation testing is positive for AS,

Table 2 Selected Genetic Syndromes With ID Plus

Macrocephaly	Microcephaly	Autism Spectrum Disorders
• *PTEN* (especially if autism present) • NSD1 or Sotos syndrome • Fragile X (frequently relative, not absolute macrocephaly) • Costello syndrome • Mutations in *RAB39B* and *OPHN1* • Lujan syndrome or *MED12* mutations	• Rett syndrome or *MECP2* mutations • AS • Rett-like disorders (*CDKL5* and *FOXG1* mutations) • Angelman-like (*SLC9A6* mutations) • Chromosome deletion syndromes (1q21, 4p, 5p, 7q11.2, 17p13.3, and 22q11.2) • PHS • Mowat-Wilson syndrome • Smith-Lemli-Opitz syndrome • Cornelia de Lange syndrome • Ataxia-telangiectasia • Cohen syndrome	• Fragile X • *PTEN* (if macrocephaly present) • Tuberous sclerosis • Rett syndrome • Chromosome deletion syndromes (16p11.2 and 15q13.3) • Mutations in *SYNGAP1, NLGN 3&4, PTCHD1,* and *GDI1* (nonsyndromic)

further analyses (deletion testing and UPD studies) are necessary to identify the precise mechanism. Elucidating the exact underlying genetic cause is essential to providing accurate recurrence-risk information and prenatal or preimplantation diagnosis (as appropriate) to family members. If the presence of a deletion is detected, high-resolution G-banded karyotype should be performed to rule out a chromosomal rearrangement. If DNA methylation studies are negative, *UBE3A* sequence analysis is the next appropriate test to perform.

Rett- or AS-like Phenotype With Negative Testing

There are several other single-gene syndromes, which can cause severe ID, microcephaly, ataxia, and seizures, and may have considerable phenotypic overlap with Rett syndrome and AS. These include Christianson syndrome (X-linked; caused by mutations in *SLC9A6*), which is characterized by seizures, ataxia, ID, microcephaly, ataxia, and a happy demeanor.[35,36] Heterozygous mutations or deletions in *FOXG1* on chromosome 14 are causative of the so-called congenital variant of Rett syndrome, with features of classic Rett syndrome but onset in the first few months of life. Mutations in *CDKL5* (also located on the X chromosome) can be associated with severe ID and early-onset seizures; a Rett-like phenotype with neurodevelopmental regression has also been reported.[37] Pitt-Hopkins syndrome (PHS) (autosomal dominant; due to mutations in *TCF4*) is characterized by severe ID with absence of speech, distinctive facial features, hyperventilation, and often, seizures or microcephaly or both; males and females with PHS can sometimes resemble patients with AS or Rett syndrome.[38] Molecular testing for all of the preceding disorders is clinically available, either as single-gene tests or as AS-like or Rett syndrome-like gene panels (offered by some diagnostic laboratories).

Prader-Willi Syndrome (PWS)

PWS should be a diagnostic consideration for infants with unexplained hypotonia and feeding difficulties, as well as older children with GDD or ID and hyperphagia leading to onset of obesity between 12 months and 6 years. Distinctive facial features, hypogonadism, and short stature are other supportive features. Seizures are seen in less than 20%, but the incidence is increased above the general population incidence. PWS may result from deletions of the Prader-Willi critical region (PWCR) on the paternally derived chromosome 15q11.2-q13 (70%-75% of patients), from maternal UPD of chromosome 15 (~25% of patients), or from an imprinting center defect (<1%) within this region.[39] DNA methylation analysis for the PWCR is the preferred first-line molecular test for PWS and has a detection rate of ~99%. Once an abnormal methylation result is obtained, subsequent testing (deletion testing and, if negative, UPD studies) is necessary to determine the exact genetic mechanism. High-resolution chromosome analysis to exclude a chromosome rearrangement is recommended for individuals found to harbor a deletion of the PWCR. As discussed earlier, determining the specific genetic etiology is critical in providing accurate genetic counseling and estimating the recurrence risks.

PTEN-Related Disorders

Heterozygous mutations in the *PTEN* gene are estimated to occur in 10%-20% of individuals with autism spectrum disorders and macrocephaly, with or without concomitant GDD or ID.[40,41] Other phenotypes that are associated with *PTEN* mutations include Cowden syndrome (CS) (a multiple hamartoma syndrome with an increased risk for benign and malignant tumors of the breast, thyroid, and endometrium; distinctive skin findings include trichilemmomas and papillomatous papules), Bannayan-Riley-Ruvalcaba syndrome (characterized by multiple hamartomatous intestinal polyps, lipomas, and pigmented macules on the glans penis; GDD or ID is present in up to 50%), Lhermitte-Duclos disease, and proteus-like syndrome. A well-established protocol exists for tumor screening in individuals with CS, and it is often recommended that patients with Bannayan-Riley-Ruvalcaba syndrome follow similar surveillance guidelines.[42] A personal or family history of *PTEN*-related tumors should prompt further consideration of *PTEN* testing in an individual with macrocephaly (OFC > 97th centile) and GDD or ID, particularly if autistic features are also present. Preferred molecular testing in a patient with a suspected *PTEN*-related disorder includes sequencing and deletion analysis of the entire coding region of the gene, plus analysis of the promoter region. The lifetime cancer risk is unknown in children with autism or, GDD or ID, or both and a *PTEN* mutation who do not have a personal or family history of *PTEN*-related tumors or characteristic dermatologic lesions. However, it may be appropriate to recommend the following CS tumor screening protocols (starting at age 18) in all patients with a pathogenic *PTEN* mutation.

Gene Panels

Several diagnostic laboratories offer multigene panels for the evaluation of GDD or ID; these panels are frequently focused on a particular subtype, such as X-linked ID (XLID), or GDD or ID plus distinctive features, including microcephaly, macrocephaly, seizures, or autism. The development of next-generation sequencing (also known as massively parallel sequencing) has allowed a large set of known disease genes to be analyzed simultaneously and at lower cost than if each gene was sequenced separately. It is estimated that mutations in genes on the X chromosome may account for about 10% of all cases of ID, and XLID panels including analysis of 90+ genes are clinically available.[43] Males with unexplained GDD or ID and a

family history, which is strongly suggestive of X-linked inheritance, would have the highest diagnostic yield on XLID gene panel testing. However, females with GDD or ID and a family history suggestive of X linkage may also be appropriate for XLID panel testing. Typically, XLID and other multigene GDD or ID panels are performed after CMA, fragile X DNA testing (if appropriate), and evaluation by a clinical geneticist have been unrevealing. One study of 600 families with multiple males with GDD or ID found causative mutations in 42% of families considered to have "definite" X linkage when they were analyzed using a panel of 90 known and candidate XLID genes.[44] In families where X-linked inheritance is less certain, X-inactivation studies on suspected female carriers (such as the mother of a male proband) may be useful. Inactivation of 1 of the 2 X chromosomes in females occurs in early embryonic life and is normally random. Significant deviation from a 50:50 ratio (usually defined as preferential inactivation of either the paternally inherited or the maternally inherited X chromosome in >75%-80% cells) may occur when a female is a carrier of a deleterious X-linked mutation. Skewed X-inactivation may also occur by chance or, more rarely, as an isolated dominant trait; thus, the presence of the skewed X-inactivation in the mother of an affected child suggests, but does not prove, that she is a carrier of an XLID disorder.[45] However, maternal X-inactivation studies, which are clinically available, can be a powerful tool when assessing the utility of pursuing multigene XLID panels.

Future Directions: Whole-Exome Sequencing (WES) or Whole-Genome Sequencing (WGS)

Advances in DNA sequencing technology have permitted not only the development of multigene panels, but also more recently, have allowed genome-wide analyses to move from the research to the clinical arena. These analyses include WES, which refers to the sequence analysis of the areas of the genome that are primarily protein-coding (about 1% of the total genome), and WGS, where the majority of all DNA sequences in a person's genome are analyzed. WES has the advantage of being cheaper and less labor intensive than WGS and is currently offered by several clinical laboratories. In some cases, WES may be targeted to certain genes of clinical interest rather than the exons of the entire genome as a whole. WES and WGS both offer the possibility of identifying a causative genetic mutation in an individual with GDD or ID for whom conventional genetic testing (CMA or single-gene analyses) has been unrevealing. "Trio" analysis, in which an affected child's exome sequence data are compared with those of his or her unaffected parents to identify de novo changes in known or candidate genes for GDD or ID, has proven to be particularly powerful in uncovering genetic diagnoses. One recent trio study of patients with nonsyndromic ID identified de novo mutations in known or candidate ID genes in 6 of 10 individuals using WES.[46] The American College of Medical Genetics has, in its 2012 policy statement, recommended consideration of WES or WGS in individuals with a likely genetic disorder for whom previous testing has failed to elucidate a diagnosis or with a condition that is known to have considerable genetic heterogeneity.[47]

WES or WGS is not without its limitations, however. First, all areas of the genome may not be captured and analyzed, even in so-called WGS, meaning that clinically significant genetic mutations may be missed. Second, epigenetic changes—heritable alterations in gene function that occur without a permanent change in DNA sequence—are not routinely detectable by either WES or WGS. The cost of WES (currently ~$4000-10,000) may be prohibitive to patients and families, although it may be covered by medical insurance in certain cases. It is likely that the cost of WES or WGS will decrease in the future as DNA technology continues to advance.

Genome-wide analyses also present significant challenges to laboratories, clinicians, and families. Large-scale sequencing is likely to uncover multiple alterations of uncertain clinical significance; additionally, it is possible that an individual may be found to have a gene mutation associated with a late-onset disease for which he or she is currently asymptomatic (eg, Huntington disease and Charcot-Marie-Tooth disease) or a mutation associated with susceptibility to disease (eg, *BRCA1* or *BRCA2* cancer-susceptibility genes). Pretest and posttest counseling with detailed informed consent for patients undergoing WES or WGS is essential and should include a discussion of the type of incidental findings that may be generated, and that results will or will not be disclosed.

Conclusion

Advances in molecular and cytogenetic technologies have resulted in changes in the recommended genetic testing approach for children with GDD or ID. The most significant updates include the inclusion of CMA as a first-tier diagnostic test, the availability of multigene panels, especially for individuals with suspected XLID, and the increasing availability of WES. Any genetic testing approach should be individualized for a child's specific clinical history, physical examination findings, and family history. Collaboration with clinical geneticists may be helpful in determining the optimal test strategy, particularly when progressing beyond first-tier analyses, and in interpreting abnormal results.

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