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REVIEW ARTICLE

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Investigating intellectual disability

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3 Key Points

1. Advances in gene testing technologies mean that a specific genetic diagnosis is now achievable in about half of all children with moderate to severe intellectual disability
2. Achieving a specific diagnosis for intellectual disability provides a range of benefits to patients, their families and their treating paediatricians.
3. Chromosome microarray, Fragile X testing, and urine metabolic screening should be performed in all children presenting with intellectual disability.

MCQs

1. **What is the most common genetic mechanism underlying intellectual disability?**

A. Chromosomal aneuploidy

B. *De novo* mutation in an autosomal dominant gene

C. Inherited mutation in and autosomal dominant gene

D. Inherited mutations in an autosomal recessive gene

E. Inherited mutation in an X-linked gene

2. **Which test has the highest diagnostic yield in the investigation of intellectual disability?**

A. Brain MRI

B. Chromosome microarray

C. Fragile X testing

D. Urine metabolic screen

E. Whole genome sequencing

3. **The diagnostic yield of genetic testing is lower in children with autism than in children with syndromic forms of intellectual disability. What is the main reason for this observation?**

A. Genes that cause autism have not yet been discovered

B. Most autism is not genetic

C. Most autism is caused by copy number variants that are not reported by testing laboratories
D. Most autism is caused by epigenetic abnormalities that are not detected by existing genetic tests
E. Most autism is caused by the interaction of multiple common gene variants that are not analysed by existing genetic tests

Answers to MCQs

Q1: B

Evidence from genomic studies has shown that more than 40% of children with intellectual disability have a *de novo* pathogenic variant in an intellectual disability gene. All other genetic mechanisms account for less than 10% of cases. The exception is when parents are consanguineous: in this situation the most common mechanism is autosomal recessive inheritance.

Q2: E.

Whole genome sequencing has the highest diagnostic yield of currently available tests, approaching 50%. Whole genome sequencing is slightly superior to whole exome sequencing. The diagnostic yield of chromosome microarray is approximately 15 % and Fragile X testing and urine metabolic screen each have a diagnostic yield <1%. Brain MRI can provide important diagnostic clues, particularly where abnormal neurological signs are present, but infrequently provides a specific diagnosis.

Q 3: E

The overall contribution of genetics to the causation of autism is thought to be similar to that of intellectual disability. Although there is considerable overlap between genetic causes of autism and intellectual disability, most autism is thought to be caused by the interaction of large numbers of common genetic variants that are inherited from both parents. In contrast, intellectual disability is more commonly caused by a single rare gene variant that has a major effect on neurodevelopment.

Abstract

The search for causation is a key component of the assessment of the child with intellectual disability. Historically, a specific diagnosis has been achievable in only a small minority of these children, but over the last decade this has changed dramatically such that a specific diagnosis is now achievable in about half of all children with intellectual disability. This improvement has been driven by major advances in genetic testing technologies, the most important of which are chromosome microarray and whole exome sequencing. Simultaneously, these technological advances have revealed many new genetic syndromes that had previously escaped clinical recognition, and demonstrated that the majority of severe intellectual disability is caused by pathogenic gene variants that arise *de novo* in the child. Although access to genomic testing is currently limited, evidence from health economic studies suggests that this testing is most cost-effective when performed early in the patient's diagnostic journey.

Introduction

Intellectual disability (ID) affects approximately 1% of the world's population, so the child newly diagnosed with intellectual disability is encountered commonly in paediatric practice. Most children with ID are identified initially because of delay in reaching multiple developmental milestones. Although a formal diagnosis of ID is only possible once a child is about 6 years old, most paediatricians will be confident in diagnosing significant cognitive impairment before this age; in this review, use of the term ID will include these younger children.

Intellectual disability is highly heterogeneous, encompassing syndromic and non-syndromic forms; severity ranges from mild to profound. Although environmental causes of ID remain important, for example fetal alcohol syndrome and congenital viral infection, it is now clear that genetic factors account for the majority of cases.[1] In general, a specific genetic cause is more likely to be detected in more severe and syndromic forms of ID than in mild and non-syndromic forms, yet the diagnostic approach is similar across ID categories.

Families of a child with suspected ID have some key questions: why did this happen, will it happen again, and what does the future hold for my child? These questions can only be answered accurately if a specific cause for ID can be identified. Thankfully, the field has seen dramatic advances in the last decade, such that the proportion of children with ID in which a specific genetic cause can be identified has increased tenfold, from around 5% to 50%. This improvement has been driven by two particular genetic testing technologies: chromosome microarray (CMA) and next generation DNA sequencing (NGS). I will review the currently available tests and outline a diagnostic approach to children with ID.

Why seek a diagnosis?

There are multiple different benefits from determining a specific aetiology for ID, although each family will have their own reasons for seeking a diagnosis.[2] A specific diagnosis provides families and paediatricians with information about expected natural history, and avoids the need for other expensive and invasive tests. For many disorders, condition-specific management and surveillance guidelines are available. A diagnosis also provides information about risk of recurrence in future pregnancy and, where necessary, the options of prenatal diagnosis and pre-implantation genetic testing. The provision of condition-specific family support is another benefit that is valued highly by families, one which has been facilitated by the internet, with even families affected by the rarest of disorders able to link across the world with similarly affected families. Finally, it is the hope of many families and researchers that the new knowledge generated by identifying specific diagnoses for intellectual disability will, over time, translate into novel and condition-specific treatments. Although the traditional view has been that ID cannot be cured, because neurological processes that lead to ID are unlikely to be reversible and are difficult to target, the elucidation of biological processes underlying genetic forms of ID has generated new optimism.[1, 3]

Clinical assessment

Such is the power of genomic technologies that it may no longer be true that history and examination are the most important part of the diagnostic evaluation for ID. Nonetheless, careful clinical assessment remains an important initial step. On clinical history, important features to note include details of pregnancy, birth, and neonatal period, and developmental history. A three generation pedigree can help identify X-linked or other familial forms of ID. Specific enquiry should be made about a family history of birth defects, miscarriage, neonatal death, and consanguinity. Medical enquiry should focus on organ defects (for example congenital heart disease and renal malformations), seizures, growth, hearing and vision. Specific enquiry should be made about progression of symptoms or developmental regression that might point to an underlying metabolic or neurodegenerative disorder. Patterns of behaviour, autistic features, self-harm, aggression, and sleep disturbance may suggest particular genetic syndromes. Examination findings are central to determining whether the ID is syndromal or non-syndromal. Features indicative of an underlying syndromal cause include abnormal growth pattern, facial or peripheral dysmorphism, abnormalities of skin and hair, neurological signs, hepatosplenomegaly, and skeletal changes.

First tier genetic investigations: chromosome microarray and Fragile X

Chromosome microarray and Fragile X testing are recommended in all children with intellectual disability, regardless of presentation. Although Fragile X is responsible for less than 1% of ID,[4] it is important not to miss this diagnosis because of its X-linked inheritance and consequent high risk of recurrence within families. Notably, the phenotypic spectrum of Fragile X is broad and some affected children do not fit the 'classical' FXS phenotype. In

particular, girls with Fragile X may have relatively mild ID, and less severe phenotypes are also seen in male mosaic forms of FXS.[5] It is also important to note that the co-existence of congenital abnormalities with ID does not preclude the diagnosis of FXS.

Chromosome microarray has now been in clinical use for more than a decade, and has a diagnostic yield of approximately 15% in children with cognitive impairment, autism spectrum disorders and multiple congenital abnormalities.[6] Microarray will detect a broad range of chromosomal causes, including autosomal aneuploidies (such as Down syndrome), sex chromosome abnormalities, and deletions and duplications of parts of chromosomes, the later now collectively referred to as copy number variants (CNVs). Some microarrays also include single nucleotide polymorphism (SNP) genotyping and these will detect homozygosity due to parental consanguinity and some (but not all) instances of uniparental disomy (UPD).[7]

Microarray allows the detection all of the well-characterised microdeletion syndromes, such as Williams syndrome (7q11.23 deletion) and velocardiofacial syndrome (22q11.2 deletion), and has also led to the discovery of a range of new deletion and duplication syndromes that previously had escaped clinical recognition, for example Koolen de Vries syndrome (17q21.31 deletion)[8] and Kleefstra syndrome (9q34 deletion).[9] In addition, the use of microarray has led to the identification of approximately 50 CNVs also found in the general population but detected with increased frequency in individuals with neurodevelopmental conditions such as intellectual disability, autism, epilepsy and schizophrenia. These CNVs are 'susceptibility loci', increasing the risk of neurodevelopmental disorders, but in isolation not sufficient to cause a phenotype. One of

the most common is the 15q11.2 deletion, which is found in the general population at a frequency of about 1 in 250, is nearly always inherited from a clinically unaffected parent, and has an estimated penetrance for neurodevelopmental disorders of 10%.[10] At the other end of the severity spectrum is the 16p11.2 deletion, which has a high penetrance for autism and intellectual disability, is rare in the general population, and more commonly arises as a *de novo* change rather than being inherited. Other neurodevelopmental susceptibility CNVs that are common are deletions and duplications at 1q21.1, 15q13.1, 16p12.1, and 17q12.

One way of conceptualising the effect of these neurodevelopmental CNVs is that they cause a shift, towards impairment, in the normal distribution of cognition and neurobehaviour.[11] Put simply, this model hypothesizes that the CNVs are causing some impairment in all individuals who carry them; however whether this impairment results in a clinically diagnosed neurodevelopmental disorder will depend on expected cognitive abilities based on genetic background. This model is supported by studies in individuals who have the common 15q11.2 deletion ascertained from the general population, who have been found to have lower functioning on neuropsychological measures compared to population controls.[12] More generally, in the population the presence of larger CNVs has been shown to be associated with lower educational achievement, in the absence of a diagnosed neurodevelopmental disorder.[13]

Other test considerations: Metabolic investigations and brain imaging

Metabolic disorders are an important cause of ID, and their detection is paramount because they are frequently progressive and specific treatments may be available. In addition, most

are autosomal recessive, so the risk of recurrence is high. Clues on history include progression of symptoms, developmental regression, multi-system involvement, and recurrent unexplained symptoms, for example vomiting or ataxia. Urine metabolic screen, combining analysis of amino acids, organic acids and glycosaminoglycans, is the specific metabolic test that is recommended in all children with intellectual disability, and is usually accompanied by ancillary blood tests comprising thyroid function, creatinine kinase, baseline haematology and biochemistry. If the clinical presentation is suggestive of a metabolic disorder, consultation with a clinical metabolic service and additional metabolic tests are likely to be required. Examples are transferrin isoforms to screen for congenital disorders of glycosylation (CDG) and 7-dehydrocholesterol to screen for Smith-Lemli-Opitz syndrome. Many metabolic disorders can now be diagnosed by genomic testing, so in some instances a genomic test, such as exome sequencing, will offer a more cost-effective and/or less invasive approach than traditional metabolic tests.

Brain magnetic resonance imaging (MRI) is not necessary in all children with ID, but can provide important information about the cause of ID, and in some cases identify treatable lesions. Brain MRI is recommended when there is abnormal head circumference (microcephaly or macrocephaly), seizures, focal neurological signs, or developmental regression. As with metabolic testing, genomic testing may be a more cost effective way of reaching an aetiological diagnosis, particularly when brain MRI requires general anaesthetic.

Second tier genetic investigation: Genomic testing

The testing technology powering the current wave of discovery is 'next generation sequencing' (NGS). NGS allows the simultaneous sequencing of all genes in the human genome, or of a selected subset of these genes. Three types of NGS are available clinically: whole genome sequencing (WGS), whole exome sequencing (WES) and disorder specific gene panels. Although WGS is considered the 'gold standard' for NGS diagnostics, the clinical uptake of WES is greater due to lower cost and detection rates that are only slightly inferior to WGS. WES does not sequence all DNA, but targets the 1-2% of the human genome that comprises actual genes, and ignores the remainder of the genome. Although there are in excess of 20,000 genes in the human genome, most testing laboratories will target analysis of WES data to approximately 4,000 genes so far linked to human disorders, sometimes referred to as the 'Mendeliome'; analysis of the remaining genes falls into the realm of research. When the clinical indication for WES is ID, analysis may be focussed further to about 1000 genes known to be associated with neurodevelopmental disorders.

The advent of NGS has driven the discovery of hundreds of new genes that cause ID. As with microarray a decade earlier, this 'genotype-first' approach has led to the characterisation of many new ID syndromes, for example the gene ARID1B, which may prove to be the single most common genetic cause of ID.[14] Yet even ARID1B is thought to account for only about 1% of all cases of ID,[15] highlighting another important lesson from genomic testing: the extreme genetic heterogeneity of ID, with all genetic causes being individually rare. This emphasises that genomics will be the only viable diagnostic approach for ID, due to its ability to test thousands of genes simultaneously.

Another unexpected discovery from genomic testing is the importance of new gene mutations, described as the '*de novo* paradigm of intellectual disability'.[1] Of all genetic diagnoses made in children with ID, the vast majority are *de novo* autosomal mutations, with autosomal recessive and X-linked inheritance accounting for minor proportions. The detection of *de novo* mutations is greatly facilitated by conducting a 'trio' analysis, whereby the affected child and both parents are sequenced at the same time. In the largest study to date, 42% of children with neurodevelopmental disorders were found to have a causative *de novo* mutation,[16] with neurodevelopmental disorders due to *de novo* mutations being present at a population prevalence of 1 in 295 births, equating globally to 140 million births per annum. The risk of *de novo* mutations increases with paternal age, such that for fathers aged above 45 years, the risk is closer to 1 in 200.[16] The detection of a *de novo* gene mutation has important implications for reproductive counselling, as the risk of recurrence in a future child is very low, thereby restoring reproductive confidence for the couple.

Although autosomal recessive genes appear to play a minor role at a population level, consanguineous relationships are an important exception. When the parents are related, 90% of diagnoses revealed by WES are autosomal recessive,[17,18] reinforcing the appropriateness of advising a 25% risk of recurrence in this setting.

The diagnostic yield of WES in children with ID will depend on patient selection, and is likely to be highest in children with severe ID and syndromic features. Early studies of WES in individuals with ID suggested a diagnostic yield of around 30%, [19,15,20] but is now approaching 50%,[16] with improvements due to improved testing technology, as well as to the steady increase in the number of known ID genes.

Although WES is a relatively expensive test, costing between AUD$1500 and AUD$4000 depending on the level of analysis required, it has been shown to be cost-effective, particularly when applied early in the patient's diagnostic journey,[21,22] emphasising the case for improved access. Unfortunately, at the time of writing, there is no consistent approach to public funding of WES in Australia, and access is inequitable. In some circumstances, families are required to self-fund testing in their child.

Genetic testing of the child with autism

The genetic architecture of autism overlaps with that of ID, and some children with autism have *de novo* gene mutations in the same genes that have been recognised to cause ID.[23] However a significant proportion of autism is caused, not by a single genetic factor, but by the presence of multiple common and inherited gene variants which combine in a particular child to cause autism.[24] This polygenic inheritance of idiopathic autism cannot be solved by existing genomic tests and, as a result, the yield of genetic testing is lower in children with idiopathic autism compared to those with ID, with CMA and WES each yielding a diagnosis of <5% in uncomplicated cases.[25,26] However when autism is accompanied by co-morbidities such as physical disability, severe ID, birth defects, or dysmorphism, the yield of genetic testing is expected to approach that of children with ID.[26] Fragile X testing should always be performed in children with autism and ID.

Future prospects

It is expected that genetic testing technologies will continue to converge, as we move towards a model where a single genetic test will be performed early in the patient diagnostic journey. Provided that costs come down, WGS is likely to replace both microarray and WES, with CNV analysis being performed using WGS data.

Despite the dramatic advances of the last decade, even the best available genetic technology is able to make a diagnosis in only half of children with severe ID. For the next decade, a key task is to determine the causative mechanisms in the remaining 50%. One possibility is that these children have causative genetic variants that are not detected by NGS, such as large scale genomic rearrangements or trinucleotide repeat expansions. Alternatively, the answer for these patients may lie within the existing NGS sequence data, but in genes that are yet to be associated with ID, or in regulatory regions whose role has not yet been recognised. Other possibilities are that these are 'mosaic' mutations which are depleted or absent in blood, or that epigenetic changes not detected by NGS are the cause. Finally, despite the focus on genetics, we should not ignore the environment, for it may be that undiscovered non-genetic causes also prove to be important.

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Figure 1: Flowchart of diagnostic pathway for investigation of global developmental delay and intellectual disability

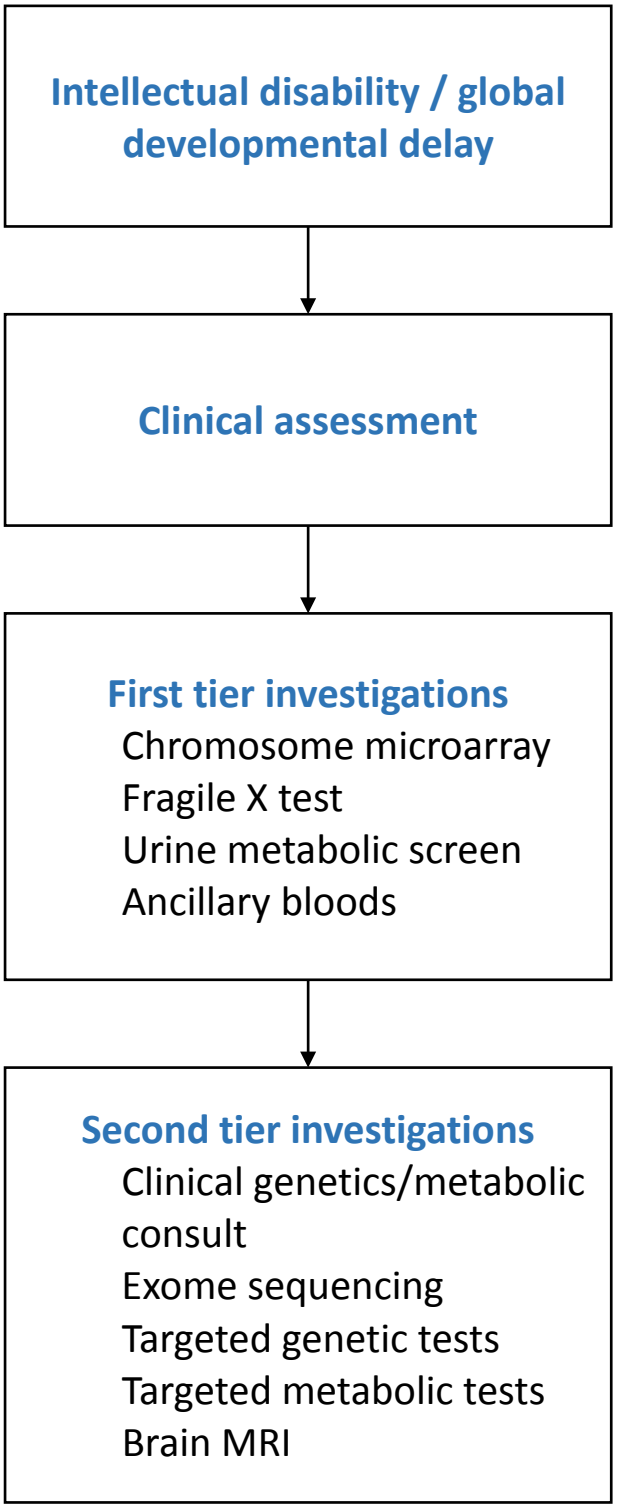

Figure 2: Diagnostic yield of common investigations in the child with global developmental delay or intellectual disability