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Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

CONTENTS

- Who/when to test
- What tests to order
- Case studies



Genetic testing in children with developmental delay

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Australian
Doctor.



Genetic testing in children with developmental delay

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Introduction

Autism spectrum disorder (ASD) and developmental delay/intellectual disability (DD/ID) are common paediatric problems, affecting about 1:30 and 1:150 children, respectively.

Establishing the cause of a child's disability is important because a specific diagnosis may assist in prognosis, counselling parents as to the risk of other children being similarly affected, and identification of therapeutic and educational interventions.

In addition, finding a cause may help avoid unnecessary and expensive further investigations. A diagnosis may help the family understand and adapt to the situation and provide an opportunity for them to establish contact with disorder-specific support organisations and/or families with the same disorder.¹

The initial assessment of a child with such a disability should include thorough history-taking and clinical examination (see table 1). However, for many children the cause of their ASD/DD/ID will remain unexplained, and targeted investigations are needed to identify the underlying cause.

Non-genetic causes of ASD/DD/ID are many but will not be the subject of this article. This issue of *Common Sense Pathology* will focus on investigations directed at identifying a genetic cause of these conditions.

Neonatal screening for metabolic disorders

The neonatal screening test aims to prevent metabolic intellectual disability and has been available for babies born in Australia since the late 1960s. Initially, the test detected a single disorder – phenylketonuria. Over the following 30 years, congenital hypothyroidism, galactosaemia and cystic fibrosis were added to Australian programs.

In the late 1990s and early 2000s, “expanded” neonatal screening using a complex technology called tandem mass spectrometry was introduced. Tandem mass spectrometry screens for more than 30 different metabolic disorders, many of which can be associated with ASD/DD/ID. About one in 500 newborn babies have a metabolic condition detected by screening.

A small proportion of infants miss screening (eg, parents refuse consent or the child is lost to follow-up after early hospital discharge) and this can be determined by contacting the screening program directly.



Newborn hearing test

The prevalence of congenital bilateral hearing loss is about one per 1000 live births. Before the introduction of the newborn hearing test, in Australia the average age at diagnosis was more than two years of age. This is suboptimal since speech, language and educational outcomes are better when intervention begins before six months of age. By early 2011, the test, which can be performed from as early as 12 hours after birth, will be available in all Australian states.

The newborn hearing test has some limitations. It misses some cases of congenital hearing loss, in particular mild hearing loss or hearing loss outside the main speech frequencies. Also, hearing loss may develop outside the neonatal period. If a child manifests signs or symptoms of hearing loss (eg, delayed speech, poor school performance or suspected intellectual disability) the child's hearing should also be tested.

Case 1

A seven-day-old newborn is recalled because of an abnormal neonatal screening test for congenital hypothyroidism.

Question 1: What additional history and examination are important?

The mother says she has the “perfect baby”, who wakes 5-6 hourly to feed and then settles back to sleep immediately. The baby passes fewer bowel motions than other babies and her mother





Table 1. Important aspects of history taking and clinical examination in children with ASD/DD/ID

Maternal History

- learning/educational achievement
- medical illnesses/conditions
- pregnancy loss¹
- neonatal deaths

Paternal History

- learning/educational achievement

Family History

- minimum three-generation pedigree
- consanguinity
- pregnancy loss¹
- developmental delay, intellectual disability, learning difficulties
- congenital abnormalities

Pregnancy History

- parental ages at conception
- teratogen exposure²
- maternal illnesses in pregnancy
- pregnancy complications
- prenatal testing³

Perinatal History

- gestation, mode of delivery, resuscitation requirements
- neonatal complications
- birth weight, length, head circumference

Child's History

- result of neonatal screening test
- result of newborn hearing screening
- developmental progress (including evidence of regression)
- behaviour⁴
- physical problems⁵
- medical conditions

CLINICAL EXAMINATION

- dysmorphism
- current weight, height, head circumference, pubertal status
- discrepant growth⁶
- features of neurocutaneous syndromes⁷ or storage disorders⁸
- assessment of vision and hearing

1. miscarriage, termination of pregnancy, intrauterine fetal death, stillbirth
2. eg, maternal alcohol and drug use, maternal infections
3. includes maternal serum screening, amniocentesis, fetal ultrasound
4. eg, stereotypies, anxiety, sleep disturbance
5. including minor anomalies eg, accessory nipple, preauricular pit, low-set ear, single transverse palmar crease, sacral dimple, clinodactyly (curvature of the little finger)
6. includes failure to thrive, short stature, microcephaly, relative macrocephaly
7. includes cafe au lait macules, axillary and inguinal freckling, hypomelanotic macules, shagreen patches, adenoma sebaceum, cutaneous neurofibromas, peripheral schwannomas, haemangioma
8. includes frontal bossing, coarse facial features, corneal clouding, skeletal abnormalities, visceromegaly and cardiac murmurs

wonders if she is constipated. The infant is hypotonic and has a large anterior fontanelle.

Question 2: What investigation(s) should be ordered?

Formal thyroid function tests and a radionucleotide thyroid scan show the baby has congenital hypothyroidism due to agenesis of the thyroid gland.

Discussion

Congenital hypothyroidism is the most common

preventable cause of intellectual disability, with an Australian incidence of about one in 3500 live births. Symptoms include prolonged neonatal jaundice, constipation, lethargy and poor muscle tone, poor feeding, a large tongue, wide fontanelle and an umbilical hernia. Treatment with thyroxine is simple and, if started early, development is normal. If an infant presents with symptoms of untreated hypothyroidism, the Newborn Screening Programme should be contacted to check the neonatal screening test result. If clinical suspicion is high, formal thyroid function tests

(T4 and TSH) should be ordered regardless of the test result.

Chromosome analysis

Chromosome analysis can detect large chromosomal abnormalities, such as the gain or loss of a whole chromosome (see figure, right) or the gain or loss of a microscopically visible section of a chromosome. Examples include Down syndrome (an additional copy of chromosome 21), Turner syndrome (loss of an X chromosome) and cri-du-chat syndrome (a small deletion of chromosome 5).

When one of these clinically well-defined syndromes is suspected, chromosome analysis should be the first-line investigation.

Case 2

An experienced mother is worried about her five-month-old baby boy who has not made the same developmental progress as his older siblings did by the same age.

Question 1: What additional history and examination are important?

The mother was aged 42 when she conceived and had a maternal serum screening test that indicated a high risk for Down syndrome. She declined prenatal testing. The family has been travelling since shortly after the baby's birth and has not attended for well-baby checks or immunisations.

The infant has mildly delayed developmental milestones, hypotonia and facial features consistent with Down syndrome.

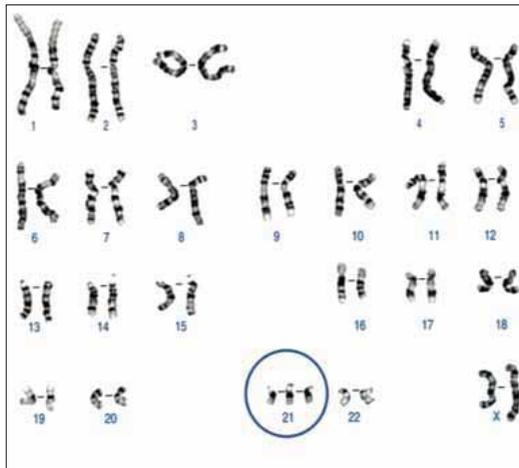
Question 2: What investigation is appropriate?

Chromosome analysis is the first-line investigation and in this case it detected three copies of chromosome 21 (trisomy 21), confirming the clinical diagnosis of Down syndrome.

The baby and his parents were referred to a clinical genetics service for genetic counselling, and the baby to a general paediatrician for management of the medical problems associated with Down syndrome.

Discussion

Down syndrome is a relatively common genetic syndrome that includes intellectual disability, characteristic facial features and a range of other health problems. In the absence of maternal serum screening programs, it is the most



A karyotype of a patient with Down syndrome showing trisomy of chromosome 21 (circled).

common chromosomal abnormality seen in children with intellectual disability.

If Down syndrome is suspected, chromosome analysis is the appropriate first investigation as this enables differentiation of Down syndrome due to trisomy 21 (three copies of chromosome 21 – 95% of Down syndrome) from translocation Down syndrome (a third chromosome 21 attached to another chromosome – 5% of Down syndrome).

For translocation Down syndrome, both parents should have their chromosomes analysed because one parent may carry a chromosomal rearrangement called a balanced translocation, which is associated with a higher recurrence risk for Down syndrome in subsequent pregnancies.

Because chromosome analysis has low resolution (ie, it can detect only relatively large chromosome changes), microarray technology has replaced it as the first-line genetic investigation for most individuals with unexplained ASD/DD/ID where a clinically well-defined syndrome such as Down is not suspected (see below).^{2,3}

Fragile X syndrome test

Fragile X syndrome is the most common cause of inherited intellectual disability and the most common known cause of autism. The most common feature of fragile X syndrome is intellectual disability of variable degree. Fragile X testing should be considered in all children with ASD/DD/ID regardless of gender.





Case 3

Andrew, a four-year-old boy, has behavioural problems and delay in language. His mother is concerned.

Question 1: What additional history and examination are important?

Andrew's mother had learning difficulties at school, particularly with maths. She left school during year 10 and found work as a sales assistant. A maternal uncle has a moderate intellectual disability. Examination identifies mild dysmorphic features in Andrew (large everted ears, an elongated face and hyperflexible joints).

Question 2. What investigation is appropriate?

A molecular genetic test for fragile X syndrome identified a full mutation in the FMR1 (fragile X mental retardation 1) gene.

Question 3. What should be done next?

Andrew and his family are referred to a clinical genetics service for counselling and consideration of genetic testing of other family members ("cascade testing").

Discussion

Fragile X syndrome is caused by a genetic error in the FMR1, which is located on the X chromosome. There are two forms of the genetic error, a full mutation and a pre-mutation, and both have serious and complex implications for families. All patients with a genetic error in this gene should be referred to a clinical genetics service for genetic counselling, further testing of family members and, if requested, prenatal diagnosis.

Microarray test

Microarray is a general term used for a number of different complex technologies that are used to study the whole genome for both loss of DNA segments (deletion) and gain of DNA segments (duplication). These deletions and duplications are collectively referred to as copy-number variations. Microarray can detect copy-number variations that are too small to be seen by conventional chromosome analysis.

For patients with ASD/DD/ID, microarray detects a pathogenic copy-number variation in 15-20% of cases, more than double the detection rate of chromosome analysis. Microarray is now the recommended first-line genetic test, along with a fragile X test, for patients with ASD/DD/ID that is

unexplained after history and examination.^{2,3}

While providing a high resolution scan of the entire genome, microarray has its limitations. A normal microarray result does not exclude all abnormalities involving DNA:

- microarray does not detect fragile X syndrome; this should be ordered as a separate test in all children with unexplained ASD/DD/ID regardless of their gender;
- microarray does not detect most single gene disorders;
- microarray does not detect very small CNVs beyond the resolution of or not covered by the microarray;
- microarray does not reliably detect mosaicism;
- microarray does not detect chromosome alterations that do not involve missing or extra segments of DNA, (eg, balanced translocations – an abnormality caused by rearrangement of parts of different chromosomes). Fortunately, balanced translocations are unlikely to be the cause of ASD/DD/ID.

Case 4 (part a)

Adam had mild global developmental delay in infancy and early childhood. At five years his parents remain concerned about delayed speech and language development, poor school performance and mildly dysmorphic facial features. In the past, a fragile X test was normal.

Question 1: What should be discussed with his parents before ordering a microarray test?

Parents/carers should be counselled about the benefits and limitations of microarray including:

- the possible microarray results and their interpretation (table 2);
- family testing may be necessary to clarify the clinical significance of an identified copy-number variation;
- in rare situations, microarray identifies an unexpected genetic abnormality that does not explain the ASD/DD/ID but could cause other health problems in the future;
- microarray does not detect all genetic abnormalities and other genetic tests may be recommended.

Most genetic laboratories offering microarray testing have information sheets for clinicians and patients that cover these issues in detail.⁴ Parents should be given the patient version of these sheets.

The practitioner needs to be prepared to give comprehensible explanations to the parents and to



Table 2. Possible microarray results and their interpretation

Microarray results	Frequency	Interpretation
No CNV identified	65-70%	The cause of the patient's ASD/DD/ID remains unexplained.
Non-pathogenic CNV	5-10%	The CNV reflects the normal variation in DNA that exists between different individuals. The cause of the patient's ASD/DD/ID remains unexplained.
CNV of uncertain clinical significance	5-7%	The CNV's disease causing nature is unclear. It may or may not be the cause of the patient's ASD/DD/ID.
Pathogenic CNV	15-20%	All available evidence indicates the CNV is the cause of the patient's ASD/DD/ID.
Unexpected pathogenic CNV	<1%	The CNV is not the cause of the patient's ASD/DD/ID, but it may cause other health problems in the future. The cause of the patient's ASD/DD/ID remains unexplained.

CNV: copy-number variation; ASD/DD/ID: autism spectrum disorder/developmental delay/intellectual disability

answer the parents' concerns. Practitioners who are unsure about any relevant aspect of a genetic test should refer the child to a general paediatrician or clinical geneticist, who can provide appropriate pre-test counselling.

Question 2. How do you request a microarray test?

Since 1 May 2010, microarray has been included in the MBS for "autism, developmental delay, intellectual disability and two or more malformations". As always, the referrer should include any relevant clinical information on the request form as this information will assist the laboratory to interpret the result.

Question 3. What is Adam's microarray result?

Adam's microarray result showed a small deletion in the short arm of chromosome 16, described as del16p12.1. The laboratory report indicated this copy-number variation is pathogenic and that all available evidence indicates it is the cause of Adam's DD/ID.

Question 4: What should be done next?

There are two main issues to consider. First, what does this mean for Adam? Second, because DNA is inherited (passed from both parents to a child), are there implications for other family members? Both issues can be addressed by referring Adam to a clinical genetics service.

Case 4 (part b)

There are no concerns about Adam's three-year-old sister's development. Adam's mother left school early in Year 12 and worked as a receptionist before having her children. His father had learning

difficulties and, despite receiving extra help, spent two years in Year 8 and left school in Year 9. He works in a factory. Adam's paternal grandfather had learning difficulties at school. There is no other family history of learning difficulties or DD/ID on either side of the family.

Adam and his parents are referred to a clinical genetics service. They attend an appointment with a clinical geneticist and a genetic counsellor.

Question 5: What is the role of the clinical genetics service?

The geneticist arranges parental testing. Adam's father carries the same copy-number variation. This is not identified in his mother.

The variation identified in Adam is part of a group of newly recognised microdeletion/microduplication syndromes involving the same region on chromosome 16. Affected individuals have variable degrees of developmental disability/intellectual disability and may have other problems including autism spectrum disorder, craniofacial and skeletal features, seizures, short stature, microcephaly, psychiatric/behavioural features, hearing loss and hypotonia.

The range of possible problems, the uncertainty about Adam's future intellectual functioning and the family implications are discussed. The clinical genetics service initiates cascade family testing to determine who else in the family has inherited the copy-number variation and is at risk of having affected children. Adam and his parents are given information about the support group Unique, for children with rare chromosome disorders. The genetic counsellor contacts the family several times over the following year to



Table 3. Micorarray results and their management

Microarray Result	Recommended management
No CNV identified OR non-pathogenic CNV	Referral to a paediatrician and/or clinical geneticist for further investigation of genetic and non-genetic causes of ASD/DD/ID.
CNV of uncertain clinical significance	Referral to a clinical genetics service, who will try to clarify the significance of the CNV.
Pathogenic CNV	Referral to a clinical genetics service for counselling about the microarray result, and range and severity of problems associated with the CNV, family studies and (if required) prenatal diagnosis. Consider referral to a paediatrician for management of the medical problems associated with the condition.
Unexpected pathogenic CNV	Referral to a clinical genetics service for counselling about the unexpected microarray result and the associated genetic condition, family studies and (if required) prenatal diagnosis. Consider referral to a paediatrician or other relevant specialist for management of the medical problems associated with the condition. Consider referral to a paediatrician and/or clinical geneticist for further investigation of genetic and non-genetic causes of ASD/DD/ID.

CNV: copy-number variation; ASD/DD/ID: autism spectrum disorder/developmental delay/intellectual disability

provide ongoing support, answer further questions and completes the family-risk notification.

Conclusion

If a child presents with autism, developmental delay or intellectual disability a detailed history and examination should be undertaken. The results of the child's neonatal screening test and newborn hearing test should be checked.

For children whose condition remains unexplained, the first-line genetic investigations are a fragile X test and a microarray test, which together

will identify a genetic abnormality in 15-20% of these children. Because DNA is inherited, if genetic testing identifies an FMR1 mutation or a pathogenic copy-number variation, the managing clinician needs to consider the implications of the finding for other family members.

The interpretation of genetic test results can be difficult and may involve testing parents and other family members. Clinical genetics services are uniquely placed to assist with test interpretation, genetic counselling, risk assessment, family testing and prenatal diagnosis.

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