



REVIEW ARTICLE

Update on the investigation of children with delayed development

Natalie Silove,¹ Felicity Collins² and Carolyn Ellaway²¹Child Development Unit and ²Western Sydney Genetics Program, The Children's Hospital at Westmead, Sydney, New South Wales, Australia

Abstract: Children develop in the domains of cognition, speech and language, motor, personal skills, social skills and activities of daily living in a predictable and organised manner. Between 3000 and 9000 Australian children born in any one year may be diagnosed with global developmental delay. Paediatricians are often faced with the dilemma of 'who' and 'how' to investigate, as the yield is often considered to be low. 'Best practice' guidelines on the investigation of global developmental delay have been published, but the evidence available for the specific recommendations varies significantly and is based mostly on levels III and IV evidence (non-experimental descriptive studies and expert opinions). This paper discusses the current views and suggests a possible algorithm for clinical practice in Australia.

Key words: developmental delay; genetic; intellectual disability; investigation; paediatric.

Illustrative Case

Daniel was the first child to Caucasian parents. The pregnancy was complicated by pre-eclampsia at 35 weeks gestation; his birthweight was 2.13 kg. His parents were concerned because from 4 months of age he was 'floppy' and motor milestones were delayed. At age 21 months, his paediatrician noted hypotonia and double-jointed thumbs. Developmental assessment noted moderate global developmental delay, with more significantly impaired speech and language. Daniel was referred for intervention including speech and language therapy, physiotherapy and occupational therapy. Hearing and vision were normal.

Key Points

- Disorders of development are common in the paediatric population, affecting 1–3% of children under the age of 5 years.
- General practitioners and paediatricians are key in identifying and evaluating children at risk and allowing for appropriate diagnosis and assessment and early intervention. Estimates of the diagnostic yield in children with global developmental delay are highly variable (10–81%).
- Identifying the aetiology of a child's delayed and disordered development is important for several reasons, which include the needs to establish causation, predict functional impact and prognosis, alter management, influence prevention strategies, identify rare conditions that are imminently treatable and not miss conditions that may exacerbate a developmental delay.

Correspondence: Dr Natalie Silove, Child Development Unit, The Children's Hospital at Westmead, Locked Bag 4001, Westmead, NSW 2145, Australia. Fax: 02 9845 2088; email: natalie.silove@health.nsw.gov.au

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Both parents had a history of mild hypermobility as children. Initial investigations including full blood count (FBC), serum electrolytes, creatinine, liver function tests, creatine kinase (CK), thyroid function tests, vitamin B12 and folate levels were all normal.

At age 25 months, Daniel was non-verbal and using 20 signs with meaning. He looked different from his parents. He had a prominent forehead, deep-set eyes, low-set ears, a broad flat nasal bridge, an upturned nose, a wide mouth and a pointed chin. He was hypermobile and had flat feet, normal strength and deep tendon reflexes. Stereotypic hand-flapping movements and hyperventilation when excited were noted.

Further investigations included a normal urine metabolic screen and serum transferrin isoforms. A submicroscopic deletion of chromosome 1p36.33 was detected by the chromosome microarray technique comparative genomic hybridisation (aCGH) (Fig. 1). Parental studies indicated this was a *de novo* finding in the child, and therefore there was a low recurrence in any future pregnancy.

In retrospect, Daniel's significant expressive speech delay, ongoing hypermobility and facial features are all typical of children who have chromosome 1p36.33 microdeletion syndrome.¹ Definite diagnosis allowed parents to give specific information to therapists and teachers. Parents were reassured that their younger child was not at risk for global developmental delay and that Daniel's problems were not related to pregnancy complications.

Definition

Children develop in the domains of cognition, speech and language, motor, personal skills, social skills, play and activities of daily living in a predictable and organised manner. All skills have a clear developmental trajectory. When the time interval between acquired milestones increases, development is

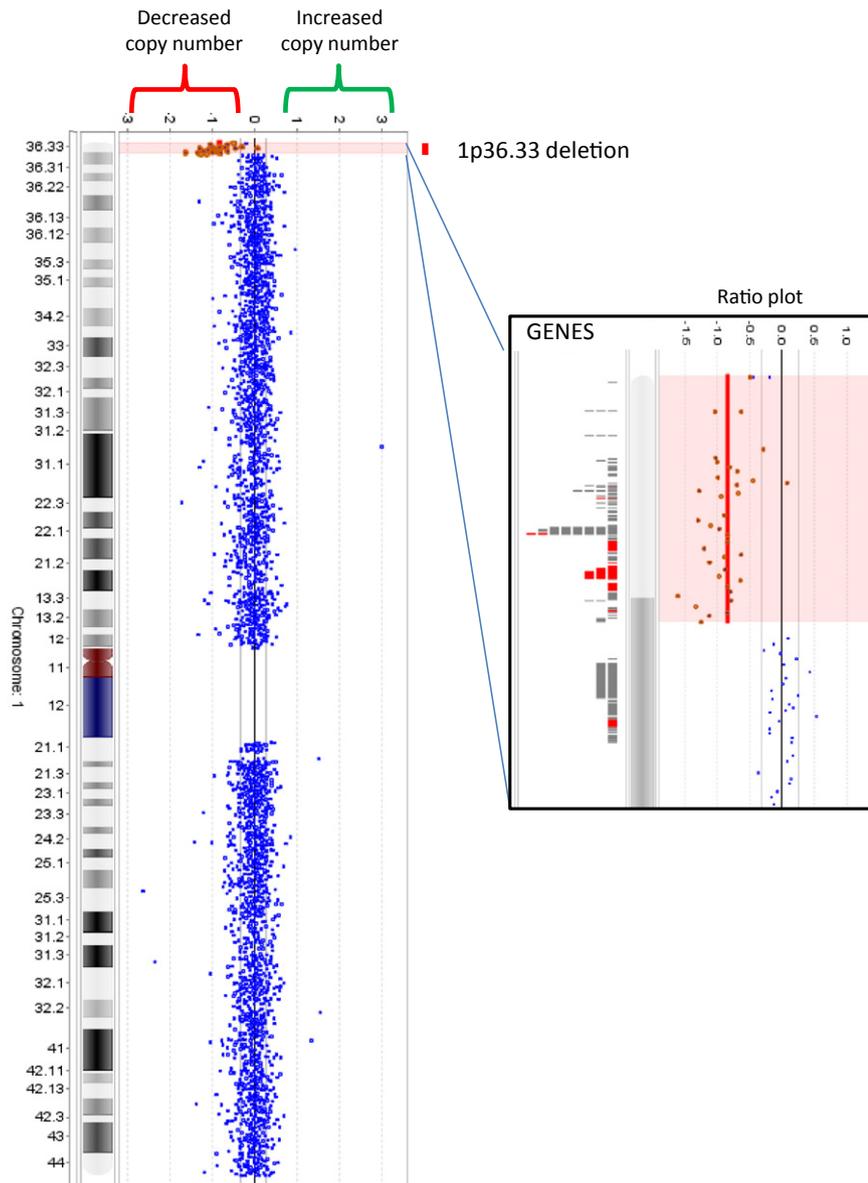


Fig. 1 Chromosome microarray data for chromosome 1p36.33 deletion. Figure courtesy of Greg Peters, Head of Microarray Testing, Cytogenetics Department, The Children's Hospital at Westmead. Modified from CGH Fusion software (infoQuant Ltd, London, UK).

'delayed'. When the acquired skills do not occur in the expected order, most often in the speech and language domain, development is referred to as 'disordered'.

A specific developmental delay is when one area of development is affected. Global developmental delay is defined as significant delay in two or more developmental domains.

Significant delay is defined as performance of two standard deviations or more below the mean on age-appropriate, standardised norm-referenced testing.² Developmental assessments include the Bayley Scale of Infant Development, 3rd edition (normed for children between the ages of 0 and 3 years 6 months) and the Griffiths Mental Development Scales Extended Revised (0–8 years). The outcome of these diagnostic assessments may be affected by other conditions, such as cerebral palsy, sensory impairments, anxiety, autism, severe neglect or environmental deprivation. The incidence of developmental

delay, particularly mild developmental delay, appears to be increased in poorer socio-economic populations,^{3–5} and this may reflect environmental factors including maternal health, smoking in pregnancy and drug and alcohol usage. A global developmental delay (particularly if mild) is therefore not always predictive of an intellectual disability.³

Intellectual disability refers to significantly sub-average intellectual ability that is accompanied by significant limitation in adaptive functioning.² General intellectual functioning is defined by the intelligence quotient (IQ) obtained by assessment with a standardised, individually administered intelligence test (usually administered over age 4 years), for example the Wechsler Intelligence Scales for Children Revised and the Stanford–Binet Intelligence Test, 5th edition. Intellectual disability is commonly divided into categories from mild to profound based on arbitrary IQ levels² (Table 1).

Table 1 Classification of intellectual disability

	IQ level
Mild intellectual disability	50–55 to approximately 70
Moderate intellectual disability	35–40 to 50–55
Severe intellectual disability	20–25 to 35–40
Profound intellectual disability	20–25

Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition Text Revision refers to mental retardation, whereas the term in Australia is ‘intellectual disability’, and in the UK, ‘learning disability’.

Prevalence

The prevalence of global developmental delay is estimated to be between 1% and 3% of children under the age of 5 years.^{6,7} The birth rate in Australia has been fairly constant at approximately 12–12.5 births per 1000 population. Based on a birth rate of 301 000 births ending June 2009 (Australia Bureau of Statistics), between 3000–9000 children born in that year may be diagnosed with global developmental delay. The majority of these children will have a mild intellectual disability.⁸

Evaluation

Identifying the aetiology of a child’s delayed and disordered development is important to establish causation, predict functional impact and prognosis, alter management, influence prevention strategies, identify rare conditions that are imminently treatable, not miss conditions that may exacerbate a developmental delay⁹ and provide accurate genetic counselling for the family.

Empiric data indicate a higher recurrence if the child is male or if the parents do not have normal intelligence; however, an overall estimate of 8–12% recurrence risk is suggested by various studies.^{10,11} Parents are also more inclined to limit their future pregnancy plans after the birth of a child with unexplained developmental delay.¹² In the future, it is possible that therapies will be developed based on an understanding of the underlying genetic cause.

Developmental surveillance requires monitoring of the child’s developmental progress over a period of time, using opportune moments such as routine physical checks, visits for immunisations or other incidental acute medical issues.¹³ Parental concerns regarding developmental delay are usually valid.¹⁴ Formal developmental screening can be performed using parental reporting measures such as the Parents’ Evaluation of Developmental Status or the Ages and Stages Questionnaire. Interactional tools, such as the Brigance Screen or the Australian Developmental Screening Test, allow direct testing by a professional. Any child identified as being at risk for developmental delay using these measures should be referred for a comprehensive developmental assessment.

Clinical Evaluation

History

- Maternal history, for example recurrent spontaneous miscarriages suggesting chromosomal rearrangement/unbalanced translocation;
- Previous stillbirths, neonatal deaths or sudden infant death may underlie an inborn error of metabolism;
- Exposure to potential teratogens, for example anti-epileptics, antidepressants, warfarin, roaccutane, alcohol (including binge drinking in the first trimester), nicotine and illicit drugs;
- Early neonatal events: complications of delivery, hypotonia, hypoglycaemia and/or seizures;
- Family history, for example parental consanguinity, history of neurological disorders, learning or developmental problems;
- Sleep disturbance and nocturnal snoring;
- Diet and pica; and
- General medical history.

Clinical examination

- Growth parameters.
- Neurocutaneous stigmata, for example tuberous sclerosis or neurofibromatosis.
- Dysmorphism, congenital abnormalities and reduced family resemblance.
- Features of storage disorders, for example hepatosplenomegaly, corneal clouding.
- Cardiomyopathy, for example mitochondrial respiratory chain disorders.
- Visual impairment, for example retinitis pigmentosa and ciliopathy disorders.
- Neurological signs.
- Vision and hearing.

Review the child with delayed development without a specific diagnosis over time to document progression and monitor for a possible emerging phenotype. Developmental regression in one or more domains requires urgent specialist referral and investigation.

Investigations

Following detailed clinical evaluation, diagnosis may be evident and laboratory investigations may be specifically targeted. In the presence of a global developmental delay that is static, non-progressive and has no clear aetiology, there is little evidence on which to base investigations. Estimates of the diagnostic yield in children with global developmental delay are highly variable (10–81%).⁶ A positive result is more likely in moderate to severe intellectual disability, compared with mild impairment.¹⁵ The cost benefit of routinely searching for a cause is difficult to establish.

There is considerable consensus between the American⁶ and UK⁹ guidelines with some notable differences, with advice differing regarding thyroid stimulating hormone and lead. The evidence available for the specific recommendations is based mostly on levels III and IV evidence (non-experimental descriptive studies and expert opinions). No Australian

consensus statement currently exists, although reviews have been published, including in this journal.¹⁶

Although there is little evidence base for studies of thyroid function with normal newborn screening, it is recommended because hypothyroidism is an easily treatable condition with significant implications if the diagnosis is missed. Many chromosomal abnormalities are associated with an increased risk of hypothyroidism, such as trisomy 21, 45X and 22q11 deletion; a small proportion of newborns with congenital hypothyroidism may be missed by newborn screening programmes, and hypothyroidism may develop in childhood. Vitamin B12 and serum ferritin are recommended, even in the absence of neurological signs or changes in FBC, if there are dietary restrictions or pica.

Measurement of CK is recommended to screen for neuromuscular disorders such as Duchenne muscular dystrophy, which can present with developmental delay before neurological deficits become obvious. Some fatty acid oxidation disorders can be associated with motor delay and elevated CK. Screening for potential teratogens, including TORCH screen, should be undertaken in any newborn presenting with abnormal neurological examination, microcephaly or hearing or visual impairment. The pickup is low, however, in the older child presenting with unexplained isolated developmental delay.

Genetic Tests

Cytogenetic-based tests

Routine cytogenetic testing (G-banded karyotype) has been the mainstay of genetic testing for the past 35 years, and chromosome abnormalities remain the commonest cause of mental retardation.¹² In the presence of mild to moderate developmental delay, a karyotype has an overall yield of 3.7–10%.¹² Some of the more common cytogenetic abnormalities identified include Down syndrome, sex chromosome aneuploidies, such as Turner syndrome (45X) and Klinefelter syndrome (47XXY), and chromosomal rearrangements such as translocations and large deletions. The recognition that the pale-staining 'tips' (telomere regions) of the chromosomes were gene-rich led to the development of more targeted chromosome analysis of these regions by techniques such as the cytogenetic-based FISH (fluorescent *in situ* hybridisation) or molecular-based MLPA (multiplex ligation-dependent probe amplification) techniques to screen for rearrangements not detectable using G-banded karyotype. Subtelomere screening is considered if there is a family history suggestive of a chromosome rearrangement and no proband is readily available for initial testing, that is, other relatives with intellectual disability, congenital anomaly, multiple miscarriages or apparent infertility. These techniques have now generally been replaced by the more comprehensive molecular karyotypes listed below, although targeted karyotype analysis using FISH or MLPA techniques may still be considered if a recognisable syndrome is clearly present, for example velo-cardio-facial syndrome (22q11deletion) or Williams syndrome (7q11 deletion). The laboratory's 'turnaround time' for these techniques is generally quicker than some of the more complex techniques listed below.

Chromosome microarray tests

New molecular-based chromosome studies – called 'chromosome microarrays' or 'molecular karyotype' – are able to screen for copy number variation (CNV) along the length of all the chromosomes to detect *deletions* and *duplications* within the whole genome. Array-based techniques such as aCGH and single nucleotide polymorphism arrays are available and are now recommended in the USA as the first-line genetic investigation.¹⁷ The diagnostic yield for individuals with unexplained developmental delay or intellectual disability is up to 15–20%.¹⁷ The limit of resolution is approximately 100 kb (0.1 Mb), compared with approximately 5 Mb for a standard karyotype. A Medicare rebate now exists for a 'molecular karyotype' such as aCGH. This may be requested by a paediatrician even if a patient has previously had a normal standard karyotype, but the two tests cannot be ordered simultaneously. Chromosome microarrays will not detect specific mutations within an individual gene or chromosome rearrangements such as balanced translocations if there is no loss or gain of chromosome material. Abnormalities detected by array-based techniques are usually confirmed by another method such as FISH or MLPA, targeted for the regions of variation noted on array. Investigation of (normal) parents with a family history of chromosome translocation or with a history of multiple miscarriages requires a standard karyotype or FISH-based study such as subtelomere FISH to exclude a balanced translocation. Some CNVs are clearly considered pathogenic (e.g. 1p36.33 deletion); others may be labelled as normal variants, as they have been reported frequently in 'normal' control samples. Interpretation becomes difficult if a genomic variant is of uncertain significance. Parental samples are often required for interpretation of variations identified in the child. If the variation is found in a normal parent, it is evidence in favour of it being benign. If the variant is *de novo* in the child, it is more likely to be significant. An increasing number of *de novo* CNVs have been associated with schizophrenia, autism and intellectual disability, for example 'susceptibility' regions on chromosome 16p11 (deletion) or 15q11 (duplication). The presence of the CNV confers a 'susceptibility' or increased likelihood of developmental problems, possibly dependent on other inherited variants and environmental factors.¹⁸

There are ethical and potential health implications when ordering chromosome microarray that may be unrelated to the cause of the developmental delay, including the potential identification of genomic variations of uncertain significance or the identification of markers of future health problems (e.g. predisposition to cancer) in the child and/or parent. Informed consent should be obtained on an appropriate consent form before testing. Appropriate consent forms are available through most tertiary paediatric institutions and may be obtained from the Children's Hospital at Westmead intranet. Chromosome microarray parent information sheets may facilitate discussion when obtaining consent and are available from various Internet sites including the Centre for Genetic Education website (<http://www.genetics.com.au/home.asp>).

Next-generation sequencing

Technology that allows cost-effective, timely sequencing of an individual's genome is being developed and includes both complete genome sequencing ('whole genome sequencing') and sequencing limited to the coding regions of genes ('exome

1. Thorough history and physical examination.
2. Formal developmental assessment.
3. Formal hearing assessment.
4. Formal vision assessment.
5. If diagnosis not apparent consider following investigations:

First line Investigations

Full blood count
 Ferritin
 Vitamin B12
 Urea and electrolytes
 Creatine kinase
 Lead
 Thyroid function tests
 Urine metabolic screen (includes amino and organic acid analyses and glycosaminoglycans screen)
 Molecular karyotype - array CGH
 DNA for Fragile X

Second line

Consider referral to neurologist or metabolic or genetic specialist for further investigations which **may** include:

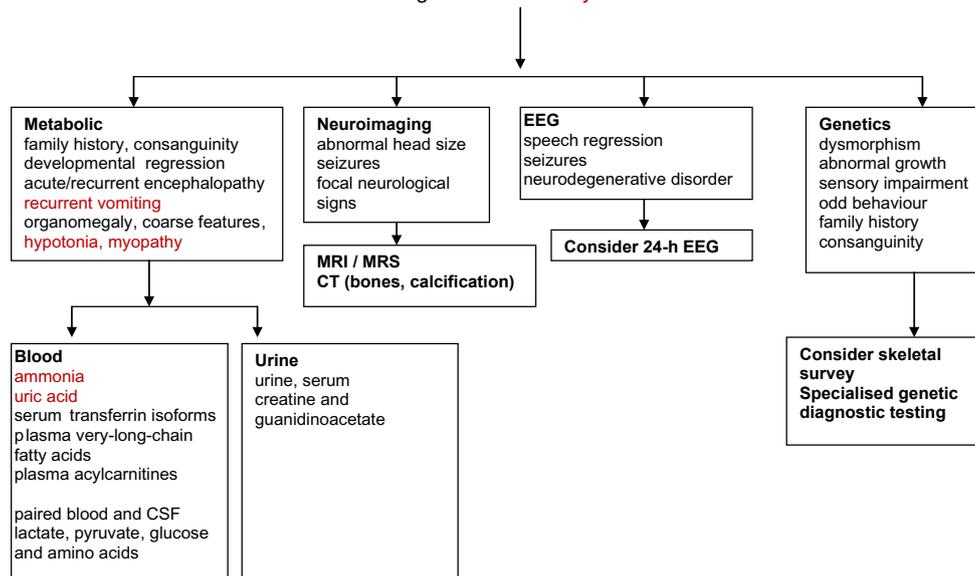


Fig. 2 A recommended approach to the investigation of children with global developmental delay of unclear aetiology.

sequencing’). These techniques have recently been shown to be powerful tools in identifying *de novo* mutations within important developmental genes in children with intellectual disability, leading to the identification of new X-linked¹⁹ and autosomal causes of intellectual disability.²⁰ These tests also have inherent ethical and health implications that are more significant than those for chromosome microarrays and are therefore likely to be available only through specific tertiary referral centres.

Fragile X studies

Fragile X syndrome is one of the commonest causes of intellectual disability, affecting approximately 1/5000 births.²¹ Primarily causing moderate intellectual disability in boys, fragile X syndrome may cause milder learning difficulties in females. The

physical features evolve with time and may not be as apparent in a younger child. Molecular analysis of the *FMRI* gene confirms the diagnosis with positive pickup in 2–6% of males and 2–4% of females with non-specific intellectual disability.¹² Given the implications for other family members, this should be considered in all cases of unexplained intellectual disability.

Other causes of X-linked mental retardation

Approximately 10% of males with intellectual disability will have a variation involving a gene on the X chromosome.¹¹ Moderate to severe disability is usually associated in males, with milder disability in females. Experience to date suggests that one gene may be associated with several different clinical phenotypes. For example, the *ARX* gene is reported (Online

Mendelian Inheritance in Man) as a cause of non-specific mental retardation, infantile encephalopathy, lissencephaly and agenesis of corpus callosum. Similarly, *MECP2* mutations can cause a varied spectrum of intellectual disability and encephalopathy. Rett syndrome should be specifically considered in females with unexplained severe global developmental delay becoming evident after a period of relatively normal development with regression such as loss of purposeful hand function and communication, with the onset of midline stereotypic hand movements.²² The clinical diagnosis is confirmed by the identification of a mutation in the *MECP2* gene. There are over 100 candidate genes on the X chromosome,¹⁹ and genetic testing is not currently available for the majority, which may not be picked up by CGH techniques. Referral to a clinical genetics service should be considered for further evaluation if X-linked mental retardation is suspected because of the significant recurrence risk.

Screening for genetic metabolic disorders

The yield from metabolic investigation of developmental delay alone is low (0.2–5%).²³ However, the diagnosis of an inherited metabolic disorder may have important implications for treatment and recurrence in future siblings. Therefore, a urine metabolic screen that includes urinary amino and organic acid analyses and a glycosaminoglycans screen should be considered as a baseline investigation in any child with developmental delay. Genetic metabolic disorders should be considered if there is a history of recurrent unexplained vomiting, specific food aversion, acute or recurrent acute encephalopathy, seizures or developmental regression. This group of disorders should also be taken into consideration if there is multisystem involvement, hypotonia, coarse facial features, organomegaly, myopathy, cardiomyopathy, liver disease or sudden unexpected death.

Neuroimaging Studies

In the presence of neurological signs or symptoms, seizures, developmental regression, microcephaly, non-familial macrocephaly and congenital malformations, imaging studies of the brain play an important diagnostic role.¹ Magnetic resonance imaging is the investigation of choice for structural abnormalities, neuronal migration disorders and evaluation of myelination. Intracranial calcification and craniosynostosis are better seen by computed tomography scan.¹ Up to 20% of patients with isolated developmental delay will have some structural anomaly, which may not in itself be diagnostic but may direct further investigation. For example, the finding of fronto-temporal atrophy may lead to further investigation of glutaric aciduria type 1. Newer techniques such as magnetic resonance spectroscopy (MRS) may be particularly useful in diagnosing certain metabolic disorders associated with intellectual disability, such as creatine transporter defects. MRS is generally only available through tertiary centres after specialist referral.

Conclusions

Paediatricians are in a unique position to ensure the early detection, evaluation and management of children with develop-

mental delay or intellectual disability. Developing consensus statements for guidelines is useful and provides an evidence-based approach to the evaluation of children with developmental delay and intellectual disability. A suggested approach is outlined in Figure 2. These recommendations refer to intellectual disability and not autism spectrum disorder (ASD). However, a significant proportion of individuals with ASD have intellectual disability, and these recommendations would then apply.

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Archie in the Snowy Mountains, by Alanna Maycock.