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VIEWPOINT

Paediatric genomic testing: Navigating genomic reports for the general paediatrician

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Monogenic rare disorders contribute significantly to paediatric morbidity and mortality, and elucidation of the underlying genetic cause may have benefits for patients, families and clinicians. Advances in genomic technology have enabled diagnostic yields of up to 50% in some paediatric cohorts. This has led to an increase in the uptake of genetic testing across paediatric disciplines. This can place an increased burden on paediatricians, who may now be responsible for interpreting and explaining test results to patients. However, genomic results can be complex, and sometimes inconclusive for the ordering paediatrician. Results may also cause uncertainty and anxiety for patients and their families. The paediatrician's genetic literacy and knowledge of genetic principles are therefore critical to inform discussions with families and guide ongoing patient care. Here, we present four hypothetical case vignettes where genomic testing is undertaken, and discuss possible results and their implications for paediatricians and families. We also provide a list of key terms for paediatricians.

Genomic testing encompasses whole-exome sequencing (WES), whole-genome sequencing (WGS) and gene panel testing. Such testing is becoming standard of care for children suspected of having a genetic basis for their intellectual disability (ID) or congenital anomalies, with diagnostic yields of up to 50% in some Mendelian cohorts.[1,2] Detection of a genetic aetiology may direct management, enable easier access to information and support, end the diagnostic odyssey, restore reproductive confidence and is also cost-effective.[3,4] Under recently established Medicare rebates in Australia, paediatricians can now order either WES or WGS for children aged 10 years or younger, with a suspected monogenic condition, based on the presence of:

- Moderate global developmental delay (GDD)/ID (criterion 1) or
- Dysmorphic facial features and one or more major structural congenital anomaly (criterion 2).[5]

Prerequisites include non-informative chromosome microarray (CMA), informed consent and consultation with a clinical geneticist. A systematic approach is recommended (Fig. 1) and has been outlined previously.[6]

Complex outcomes such as variants of uncertain significance (VUS), incidental findings and novel disease gene discovery often require a nuanced understanding of genomic principles. In addition, there is variation in report language and structure, and approaches to reporting incidental findings between accredited laboratories. In this report, we aim to assist the paediatrician with clinical interpretation of results using four hypothetical cases, supported by a listing of common genetic terms (Table 1). These scenarios are presented as an educational exercise to illustrate important genomic concepts and do not represent actual patients. The data should not be used for formal clinical or molecular genetic analyses or classification either for diagnostic or research purposes.

This project did not use any real patient cases, and hence ethics approval was not required.

Case 1

A 2-year-old boy with moderate GDD is referred to a paediatrician. He is the second child to a non-consanguineous

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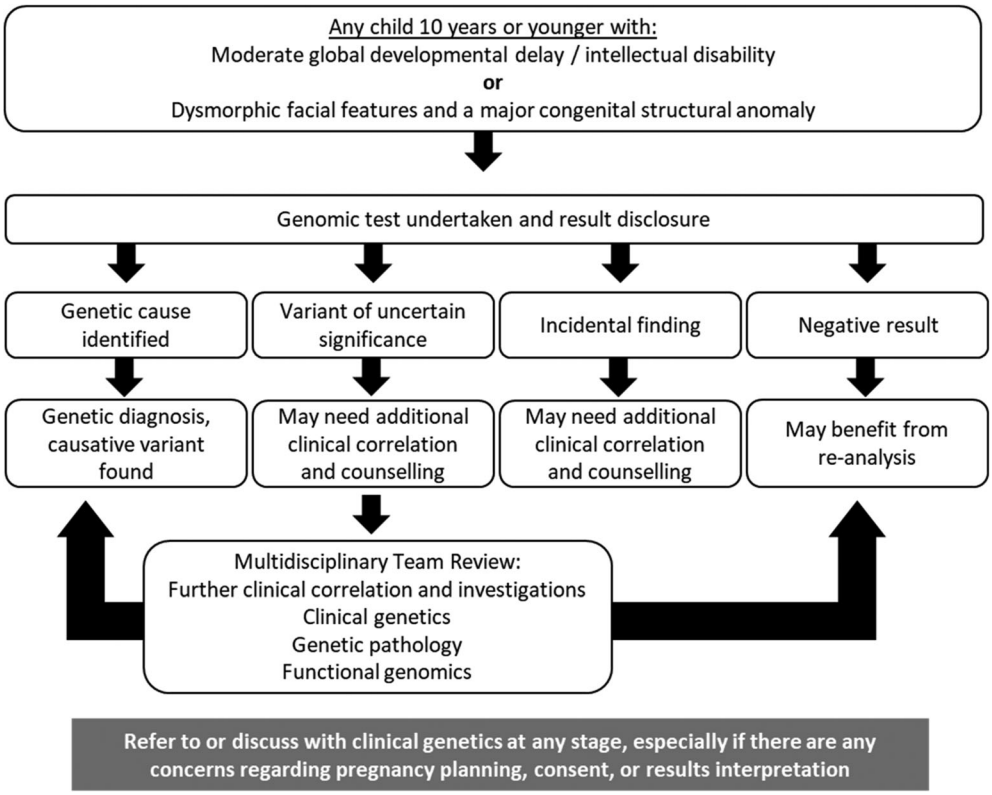

Fig 1 Possible results of genomic testing. For variants of uncertain significance, the additional clinical/genetic correlation provided in expert multidisciplinary team review meetings provides the paediatrician with additional pathways for interpreting the variant, and possibly reclassifying as pathogenic or benign. Examples of these scenarios are provided in this paper (Adapted from Sachdev *et al.*,[6] with permission).

couple, with non-contributory family history. The child has normal growth parameters, a prominent forehead and hypertelorism. He is mildly hypotonic but has no focal neurological findings.

CMA, Fragile X testing and urine metabolic screen (UMS) are non-informative. The local genetics service is consulted, and the child is eligible for genomic testing under Medicare Item Number 73359 (criterion 1). Trio WES is recommended, after family consent. This detects a *de novo* heterozygous missense variant (c.1067G>C predicted to result in the amino acid change p.Arg356Pro) in the *TGFBR2* gene. Pathogenic variants in *TGFBR2* cause Loeys–Dietz syndrome (LDS), an autosomal dominant condition associated with facial features including hypertelorism, joint hypermobility, cardiovascular complications (aneurysms and progressive aortic root dilatation), and sometimes GDD.[7]

This variant in case 1 is classified as pathogenic by the laboratory because it is *de novo*, absent from population controls, has been reported previously in multiple patients with LDS and has supportive scores from *in silico* (computational) tools.

Discussion with the local genetics service confirms the variant fits with the clinical features. On repeat examination, the child is noted to have a bifid uvula and joint hypermobility, consistent with the LDS phenotype. This instigates referral for cardiac assessment, with a plan to consider beta-blocker therapy if significant aortic root dilatation develops. This diagnosis allows the family to access condition-specific support groups and tailored allied health therapies via the National Disability Insurance Scheme. Genetic counselling advises a low recurrence risk.

Take Home Messages

- Early diagnosis of a genetic syndrome can lead to improved surveillance, changes in management and accurate recurrence risk counselling
- Diagnosis can improve access to support services and therapies
- Indicators of variant pathogenicity:
  - Phenotypic fit
  - Previously reported as pathogenic (with evidence supporting this assertion provided)
  - Absent from population controls
  - Supportive *in silico* tool scores

Case 2

A 17-month-old girl presents with atypical afebrile seizures and moderate GDD. Conception was via donor egg. The parents wish to utilise further frozen embryos from the same donor.

Table 1 Common genetic terms

Term	Meaning
>	A difference in the DNA sequence (at the indicated position) from the reference base on the left to the one on the right of the '>' symbol. For instance, *IDUA*:c.1206G>A implies that in the *IDUA* gene, there is a difference at the 1206th coding nucleotide, from guanine to adenine
ACMG criteria	A set of suggested criteria that are widely used to classify the pathogenicity of any variant identified (published by the ACMG)
Allele	A term describing the DNA sequence of one version of a gene
Autosomal dominant	A type of inheritance in which a pathogenic variant only needs to be on one copy of the chromosome to cause a phenotype
Autosomal recessive	A type of inheritance in which both copies of the chromosome need pathogenic variants to cause a phenotype
Benign	A variant that has been assessed with strong confidence (>99% likelihood) not to cause a Mendelian disease
c.	The position of a variant in the protein-coding regions of a particular nominated version (transcript) of the gene (starting from the beginning of the start codon). For instance, NM_000492.3(*CFTR*):c.1521 refers to the 1521st nucleotide within the canonical transcript of the CFTR gene
Canonical transcript	See transcript/transcription
Chromosome microarray	A genetic test that can detect copy number variants across the whole genome. The exact test specifications of the platform used determine the smallest size of copy number variation that can be detected
Controls	Individuals (often in a database) who do not have any evidence of disease
Copy number variant	A variant that is characterised by greater (duplication) or fewer (deletion) copies of a segment of the genetic code compared to the reference genome (for most of the genome, the reference copy number is 2, except for X and Y chromosomes in males)
De novo	Describes a variant that is not inherited from a parent, but is new in the patient. *De novo* variants arise at the time of egg or sperm formation, or very soon after fertilisation
Deletion	See copy number variant
Duplication	See copy number variant
Exon	The parts of a gene that code for the final mRNA (mostly protein-coding sequence), which is used as the template to make a protein. Most disease-causing variants that we currently understand are located in exons
Expressivity	The extent to which a phenotype is expressed by an individual with a genetic condition. Many genetic conditions have variable expressivity, whereby affected individuals may manifest with different clinical features and with differing degrees of severity
Functional domain	A region of the protein that has a specific role: variation from normal in a critically important functional domain may be more likely to cause disease than the one in a non-functional domain
Functional studies	Investigations undertaken to assess the function of genes and the consequences of genetic variants (e.g. biochemical tests such as enzymology)
g.	The genomic position on the relevant chromosome (starting from the top of the short arm of the chromosome). For instance, ChrX:g.1 refers to the first nucleotide on the X chromosome
Gain-of-function variant	A variant that causes an increased level of function or activity of the gene product
Genomic testing	Genetic testing that sequences and then analyses all (or parts) of an individual's entire DNA sequence (genome). This can include WES (analysis of exons and exon–intron boundaries), WGS (analysis of the whole genome) or gene panels (analysis of a set of selected genes associated with the patient's specific phenotype)
Genotype	The genetic make-up of an individual at a particular location or in a particular region
Germline	A variant that is present in all cells in the body, and occurred either in formation of germ cells, or immediately after conception or is inherited
gnomAD	A large database of individuals, derived from patients with ischaemic heart disease and mental health conditions, that is, depleted of individuals with severe childhood-onset genetic conditions. This database is helpful for ruling out pathogenicity of common population variants
Gonadal mosaicism	The possible presence of mosaicism for a particular variant in the gonadal cells of an individual
Haploinsufficiency	A gene where reduction in functioning product by 50% is expected to cause a disease or phenotype (such as if one copy of the gene has a loss-of-function variant)
Hemizygous	Present in the only copy of the chromosome (e.g. variants in genes on X chromosome for a male)
Heterozygous	Present in one copy out of the two copies of the relevant chromosome
HGVS nomenclature	A standardised nomenclature guideline that is typically used to communicate genetic information, such as variant details and genetic test results (published by HGVS, an international collaborative group that aims to foster discovery and characterisation of genomic variants)
Homozygous	Present in both copies of the relevant chromosome

(*Continues*)

Table 1 (Continued)

Term	Meaning
Hypomorphic allele	A variant that leads to reduced function of a gene product. Often used in connection with variants that are associated with mild or no phenotype
In silico tools	Also known as pathogenicity prediction software, these are computerised prediction tools using a variety of algorithms, which provide predictions as to whether some types of variants are likely to affect the function of a protein and therefore cause disease
Incidental finding	A variant, identified on genetic testing, which has health implications that are unrelated to the reason for doing the test
Intolerant of missense substitution	Regions of genetic code (or a whole gene) in which changes in the amino acid composition are assessed as more likely to significantly alter its function
Intron	The parts of a gene between exons. Introns are generally cut out in RNA processing and are not used as a template to make a protein. However, intronic segments may have other functions, and we do not completely understand these parts of the genetic code
Invariant	Genetic code (or the amino acid it codes) is identical across numerous species from simple to complex, suggesting a functional importance that is more likely to be disturbed by any variation
Likely benign	A variant that has been assessed as highly likely (>90% likelihood) not to cause a Mendelian disease
Likely pathogenic	A variant that has been assessed as highly likely (>90% likelihood) to cause a Mendelian disease
Loss-of-function variant	A variant that causes a complete absence of a gene product
Mendelian	Refers to inheritance in a pattern consistent with the principles laid out by Mendel – specifically autosomal recessive, autosomal dominant or X-linked inheritance
Missense	A variant that corresponds to a different amino acid at that position compared to the reference
Mosaicism	The presence of two or more cell lines with different genetic composition
Mutation	Same as variant, although often used in connection with variants that are pathogenic or thought to be disease-causing
Nonsense	A variant that introduces a stop codon earlier than usual and therefore leads to either no functional protein or a truncated protein being produced
Novel disease gene discovery	Identification of an association between pathogenic variants in a gene and a Mendelian disease
Null variant	A variant that causes a non-functioning gene product, or that prevents translation to form any product at all
OMIM	A freely available database of human genes and associated phenotypes
p.	The protein (amino acid) position of the variant (starting from the first amino acid, the start codon). For instance, *CFTR*: p.508 refers to the 508th amino acid in the protein produced by the CFTR gene
Pathogenic	A variant that has been assessed with strong confidence (>99% likelihood) to cause a Mendelian disease
Penetrance	A quantitative measurement, describing the proportion of individuals carrying pathogenic variants in a gene that manifest signs or symptoms of the associated condition. Incomplete penetrance describes the state where there are individuals with pathogenic variants in a gene who do not manifest the condition
Phenotype	Observable clinical traits (symptoms, signs, biochemistry, radiology, etc.) in an individual
Preimplantation genetic diagnosis	Testing of embryos (conceived via *in vitro* fertilisation) for a genetic condition
Prenatal testing	Invasive testing (often genetic) of a fetus during a pregnancy, usually from a chorionic villus sample or amniocentesis sample
Proband	The first family member where the possibility of a genetic condition is considered
Reference genome	A constructed reference of the 'normal' DNA sequence across all of a human genetic code
RefSeq	A repository that contains well-annotated reference sequences of DNA, RNA transcripts and protein
Segregation	Assessing whether the variant(s) are present in family members in a pattern consistent with the expected inheritance pattern of disease. For instance, both parents being carriers for a child with compound heterozygous variants causing an autosomal recessive disorder
Single nucleotide polymorphism	A single base variation in the genetic code that is known to be present in many individuals (and so is unlikely to be causative of a disease phenotype)
Singleton testing	Sequencing and analysis of only the patient's DNA sequence
Somatic	A variant that occurred in dividing cells postnatally and is therefore only present in some cells in the body
Transcript/transcription	The process of 'reading' DNA to RNA for subsequent processing to mature mRNA which is then translated to protein. Many genes can be transcribed in different ways, for example by using different transcription start sites, alternate splicing can also occur, with the resulting mRNA coding for different versions of a protein. The canonical transcript is generally the longest known transcript, although this may not always be the most biologically important transcript
Trio testing	Sequencing and analysis of the patient's and both biological parents' DNA sequence
Variant	A difference in the genetic code compared to the reference genome

(*Continues*)

Table 1 (Continued)

Term	Meaning
Variant of uncertain significance	A variant for which there is insufficient evidence to determine if it causes a Mendelian phenotype or is a benign variation in the genetic code
WES	See genomic testing
WGS	See genomic testing
Wild type	The most common or usual version of any particular gene
X-linked	A type of inheritance in which the phenotype is caused by pathogenic variants on the X chromosome

ACMG, American College of Medical Genetics and Genomics; HGVS, Human Genome Variation Society; mRNA, messenger RNA; OMIM, Online Mendelian Inheritance in Man; WES, whole-exome sequencing; WGS, whole-genome sequencing.

Electroencephalogram demonstrates non-specific abnormalities. Outpatient magnetic resonance imaging scan and neurology review are planned, with a waiting time of several months. CMA, Fragile X testing and UMS are non-informative.

On examination, the child has appropriate growth parameters but bilateral symmetrical hypertonia and hyperreflexia. After consultation with the local genetics service, she qualifies for a Medicare-rebatable genomic test (criterion 1). As a sample from the biological mother is not available, singleton WES is performed, identifying a heterozygous variant in the *WDR45* gene. Pathogenic variants in this gene are associated with 'neurodegeneration with brain iron accumulation' (NBIA), a condition that results in significant GDD/ID, seizures and neurocognitive deterioration in adolescence. Cerebral imaging characteristically demonstrates iron accumulation in the globus pallidus and substantia nigra.

The variant identified in this child is classified as a VUS. It is absent from controls, and *in silico* tools predict pathogenicity. A single previous report suggests pathogenicity; however, supporting evidence for this assertion was lacking in this report. Proving the variant is *de novo* would be sufficient to upgrade the classification; however, this is not possible as no maternal sample is available. Overall, there is insufficient evidence to classify the variant as likely pathogenic or pathogenic.

A multidisciplinary team meeting (MDTM) suggests expediting the magnetic resonance imaging scan, which demonstrates iron accumulation in the globus pallidus and substantia nigra, a specific finding for neurodegeneration with brain iron accumulation (NBIA). Given the characteristic radiological findings, the laboratory upgrades the variant to likely pathogenic.

It would be expected that the likely pathogenic variant is *de novo*, given the *WDR45* gene is inherited in an X-linked dominant pattern and it is unlikely that an affected individual would donate eggs. The risk of recurrence in the remaining embryos is expected to be low; the possibility of gonadal mosaicism means recurrence cannot be completely ruled out. Pre-implantation genetic diagnosis or prenatal testing options could be considered with appropriate counselling if recurrence is a concern for the parents.

Take Home Messages

- Discussion of a complex genomic result utilising an MDTM approach with referrer, clinical geneticist, genetic counsellor, genetic pathologist and scientists may be useful
- The presence of some supportive features (absence from controls, evidence from *in silico* tools and reports without supporting evidence) is not sufficient to classify a variant as (likely) pathogenic
- Additional information can help a reporting laboratory clarify whether a VUS is (likely) pathogenic or (likely) benign
- Useful information may include specific relevant phenotype information (clinical, biochemical and imaging) or proving a variant is *de novo*
- VUS generally should not be used as the basis of action (predictive testing and prenatal testing)

Case 3

An 8-year-old boy presents with moderate ID, autism and complex partial seizures. He is the youngest of three children to non-consanguineous healthy parents. CMA, Fragile X testing and UMS are non-informative. The parents are renewing his National Disability Insurance Scheme plan and feel that a diagnosis may help secure funding.

On examination, he has normal growth parameters with distinctive facial features, including a broad forehead and short nose. He has mild hypotonia with large joint hypermobility but no focal neurological signs.

The local genetics service recommends trio WES (Medicare-rebatable under criterion 1). WES identifies a maternally inherited hemizygous variant in *SLC6A8*; pathogenic variants in this gene cause cerebral creatine deficiency syndrome type 1. The variant is classified as a VUS. There is little evidence to support or exclude pathogenicity.

Cerebral creatine deficiency syndrome type 1 is an X-linked condition associated with GDD, autism and seizures, with radiological features of low or absent creatine. It may fit the boy's phenotype, however, his features are non-specific. Following an MDTM, magnetic resonance spectroscopy is performed, with equivocal results regarding evaluation of the creatine peak. As this is an X-linked condition, segregation of the variant is undertaken in the neurotypical, healthy older brother. Subsequent identification of the variant in the brother means the variant can be re-classified as likely benign and is unlikely to be causing the proband's phenotype. The family is informed that a cause has not been identified but that the genomic

data may be reanalysed 18 months later (as per Medicare Item Number 73360). This has a 10–15% of added yield,[8] due to evolving genomic technology and novel disease gene discovery.

Take Home Messages

- Testing relatives can be helpful in clarifying the significance of a VUS
- Identifying a variant(s) in a healthy relative strongly suggests that it is unlikely to be pathogenic, when the relevant condition is fully *penetrant* in childhood
- Reanalysis of existing genomic data over time has been demonstrated to provide a significant yield, and is Medicare rebatable (73360)

Case 4

A 9-year-old boy with mild ID is seen in the paediatric clinic. He has behavioural difficulties, attention-deficit/hyperactivity disorder and sleep issues. At birth, he was diagnosed with a large cleft palate, repaired successfully at 6 months of age. He is the first child of non-consanguineous parents, who are concerned about recurrence risk. Growth parameters are on the third centile and the child is hypoteloric, with a short nose, low-set ears and 2–3 toe syndactyly.

CMA, Fragile X testing and UMS conducted at age 3 were non-informative. The local genetics service recommends trio WES (Medicare rebatable under criterion 2), which identifies a single heterozygous pathogenic variant (c.1210C>T, with predicted amino acid substitution p.Arg404Cys) in *DHCR7*. Bi-allelic pathogenic variants in this gene are associated with the autosomal recessive condition Smith–Lemli–Opitz syndrome (SLOS). This variant is classified as pathogenic, having an extremely low population allele frequency, compatible with a variant implicated in autosomal recessive disorder, and being reported multiple times in patients with SLOS. However, a second pathogenic variant (necessary to cause a recessive disorder) is not identified, despite the strong phenotypic match. SLOS is associated with raised levels of 7-dehydrocholesterol, which is confirmed in this child (1005 μmol/L; reference range < 2.5 μmol/L).

The case is discussed in an MDTM with metabolic, genetic and genomic expertise. It is noted that some copy number variants may be too large to detect with WES and too small to detect with older CMA technologies. WGS can detect small exonic deletions, however, it is not readily available. A newer, higher-resolution microarray detects a pathogenic deletion of four exons within *DHCR7*; this is confirmed on parental segregation testing to be on the opposite allele to the missense variant, and provides molecular confirmation of SLOS. The family is counselled regarding reproductive options, as there is a 25% recurrence risk for autosomal recessive conditions.

Take Home Messages

- WES does not detect:
  - (all) deletions and duplications (copy number variants)
  - trinucleotide repeat disorders (e.g. myotonic dystrophy, Fragile X syndrome)
  - methylation abnormalities (e.g. Angelman syndrome or Prader–Willi syndrome)
  - mitochondrial DNA disorders
- Referral to the local clinical genetics service can be helpful to identify alternative molecular bases for disease

Discussion

The above-mentioned cases demonstrate the complexities of interpreting genomic test results. Discussion with local genetics services may be requested, and MDTM review of clinical phenotypes and variants, including additional expertise from subspecialists, can assist in streamlining interpretation, management and counselling.

As seen in case 1, identification of a genetic variant explaining a patient's phenotype has multiple benefits. From a clinical perspective, diagnosis may lead to disease-specific surveillance and therapies. Tan *et al*. noted that a molecular diagnosis changed clinical management in 26% of paediatric patients.[9] For this case, a diagnosis of LDS directed early commencement of beta-blocker therapy which could help avoid or delay invasive aortic root surgery.[7,10]

From a psychosocial perspective, a molecular diagnosis ends the diagnostic odyssey, allowing access to support groups and funding.[9] Accurate recurrence risk estimation also assists family planning. Given the variant in this case was *de novo*, the risk in future pregnancies would be low but not zero, due to the possibility of gonadal mosaicism (<1% for most genes, but up to 1–4% for some).[11,12]

Once variants of interest are identified, laboratories use a set of criteria, such as those of the American College of Medical Genetics and Genomics (ACMG)[13] to determine if the variant is pathogenic (disease-causing) or benign (normal variation). A combination of multiple pieces of evidence is required to classify a variant, which may include population data, gene-specific knowledge, segregation, computer predictions, clinical information and functional data. Several of these lines of evidence were present in this case, facilitating classification of the variant as pathogenic.

Trio WES or WGS is generally recommended because parental sequencing data assist with filtering and analysis of identified variants, with a higher likelihood of diagnosis.[14] If a variant is inherited from a healthy parent, it is unlikely to cause a highly penetrant autosomal dominant condition. However, if one or both biological parents are not available, as in case 2, singleton WES or WGS is covered if the Medicare inclusion criteria are met.

Case 2 highlights that a VUS can be reclassified by utilising clinical information if the condition associated with a gene fits the patient's phenotype. Thorough clinical phenotyping (history, examination and relevant investigations) is therefore critical for interpreting genetic tests.[15] Neuroimaging findings, examination features or biochemical profiles may provide supportive evidence, with non-specific features such as GDD or autism of less use. It is often helpful to discuss these points in an MDTM. In case 2, the

highly characteristic neuroimaging findings allowed the laboratory to upgrade the variant to likely pathogenic.

Functional studies can provide further evidence. However, functional assays used in research studies may not be validated to a clinical standard and should be interpreted with caution. There are relatively few genes for which robust, clinically validated functional studies are available.

Likely pathogenic and pathogenic variants are typically considered actionable results. In case 2, the final classification of the variant as likely pathogenic helped inform the family of their reproductive options. However, if the variant had continued to be classified as a VUS, the patient's diagnosis may have been suspected but not proven. A VUS should not be used for predictive testing in asymptomatic/unaffected individuals, nor in prenatal testing, without discussion with the local genetic service.

For any variant, assignment of pathogenicity requires multiple different lines of evidence; in case 3, insufficient evidence was available. The VUS was in *SLC6A8*, which is associated with an X-linked recessive condition. As female carriers of X-linked recessive conditions are generally clinically unaffected, the finding that the mother carried the variant neither supported nor refuted pathogenicity. Unfortunately, definitive phenotypic information to clarify whether an *SLC6A8*-related disease fit the patient's phenotype was not provided by magnetic resonance spectroscopy. For X-linked recessive conditions with full penetrance at a young age, testing unaffected male relatives may be helpful when the mother is a carrier. In this case, the unaffected brother was tested and had the same variant; this made it unlikely the variant was pathogenic, and therefore unlikely to cause the patient's phenotype.

Uninformative results like these may occur because the cause of the condition is not monogenic, or because current genomic methodology cannot identify the underlying genetic cause, or that the causative gene has yet to be linked to human disease. Furthermore, certain genetic conditions cannot be detected by WES, and alternative genetic tests may be appropriate. For example, triplet repeat expansions, the main cause of Fragile X syndrome and accounting for about 1% of ID, are not detectable by WES.[16,17]

Case 4 illustrates that certain variant types are difficult to detect on WES. Most technologies used for WES produce short sequence reads (up to a few hundred base pairs); hence, a larger deletion or duplication may not be detected.[18] Consequently, it is important to liaise with clinical genetics and the molecular laboratory, especially when there is a high index of suspicion for a monogenic diagnosis. In this case, the clinical phenotype of cleft palate, facial dysmorphism, 2–3 toe syndactyly and growth restriction was consistent with the autosomal recessive condition SLOS[19] and would warrant further investigation, even though only one pathogenic variant was detected on WES. Again, the clinical phenotype is critical in guiding genetic test interpretation and any further relevant (genetic) investigations.

The reproductive risk for this couple with each subsequent pregnancy is 25%. Identifying the specific molecular basis for this autosomal recessive disease enables the couple to consider prenatal testing, or alternatively preimplantation genetic diagnosis, if they wish.

Conclusion

Integration of genomic testing into paediatric practice is becoming standard of care, with the availability of a Medicare rebate for certain clinical presentations, high diagnostic yield and clear benefits for patients and families. However, correct interpretation and clinical response to results are critical, particularly for a VUS. A general understanding of genomic language, variant classification and the importance of careful phenotyping will aid paediatricians in interpreting and explaining genomic results to families and in formulating management plans. Results may be complex, and therefore a low threshold is recommended for accessing local genetics service support. Where possible, an MDTM approach involving the referrer, clinical and laboratory genetics, genetic counsellors and subspecialty expertise is an optimal model to address complex results.

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References

1 Stark Z, Tan TY, Chong B *et al*. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet. Med.* 2016; **18**: 1090–6.
2 Vissers LELM, van Nimwegen KJM, Schieving JH *et al*. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. *Genet. Med.* 2017; **19**: 1055–63.
3 Yeung A, Tan NB, Tan TY *et al*. A cost-effectiveness analysis of genomic sequencing in a prospective versus historical cohort of complex pediatric patients. *Genet. Med.* 2020; **22**: 1986–93.
4 Palmer EE, Schofield D, Shrestha R *et al*. Integrating exome sequencing into a diagnostic pathway for epileptic encephalopathy: Evidence of clinical utility and cost effectiveness. *Mol. Genet. Genomic Med.* 2018; **6**: 186–9.
5 Medicare Benefits Schedule. *Category 6 Pathology Services 73358, 73359 and 73360*. Canberra: Commonwealth of Australia; 2007. Available from: http://www9.health.gov.au/mbs/search.cfm?q=exome&Submit=&sopt=S [accessed 11 November 2020].
6 Sachdev R, Field M, Baynam G *et al*. Paediatric genomic testing: Navigating Medicare rebatable genomic testing. *J. Paediatr. Child Health* 2021; **57**: 477–83.

7 Loeys BL, Chen J, Neptune ER *et al*. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in *TGFBR1* or *TGFBR2*. *Nat. Genet*. 2005; **37**: 275–81.
8 Tan TY, Stapleton R, Stark Z *et al*. Evaluating systematic reanalysis of clinical genomic data in rare disease from single center experience and literature review. *Mol. Genet. Genomic Med.* 2020; **23**: e1508.
9 Tan TY, Dillon OJ, Stark Z *et al*. Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. *JAMA Pediatr.* 2017; **171**: 855–62.
10 MacCarrick G, Black JH, Bowdin S *et al*. Loeys-Dietz syndrome: A primer for diagnosis and management. *Genet. Med.* 2014; **16**: 576–87.
11 Biesecker L, Spinner N. A genomic view of mosaicism and human disease. *Nat. Rev. Genet*. 2013; **14**: 307–20.
12 Rahbari R, Wuster A, Lindsay S *et al*. Timing, rates and spectra of human germline mutation. *Nat. Genet.* 2016; **48**: 126–33.
13 Richards S, Aziz N, Bale S *et al*. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* 2015; **17**: 405–23.
14 Clark MM, Stark Z, Farnaes L *et al*. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom. Med.* 2018; **3**: 16.
15 Baynam G, Walters M, Claes P *et al*. Phenotyping: Targeting genotype's rich cousin for diagnosis. *J. Paediatr. Child Health* 2015; **51**: 381–6.
16 Coffee B, Keith K, Albizua I *et al*. Incidence of fragile X syndrome by methylated FMR1 DNA. *Am. J. Hum. Genet*. 2009; **85**: 503–14.
17 Kraan CM, Godler DE, Amor DJ. Epigenetics of fragile X syndrome and fragile X-related disorders. *Dev. Med. Child Neurol*. 2019; **61**: 121–7.
18 Teo SM, Pawitan Y, Ku CS *et al*. Statistical challenges associated with detecting copy number variations with next-generation sequencing. *Bioinformatics* 2012; **28**: 2711–8.
19 Nowaczyk MJM, Wassif CA. *GeneReviews: Smith-Lemli-Opitz Syndrome*. Seattle, WA: GeneReviews; 1998. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1143/ [updated 2020, accessed 11 November 2020].

The colourful parrots by Louis Csirszka (age 7) from Operation Art 2021