A Diagnostic Odyssey – Red Flags in the Red Sand

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There are 5,000-8,000 known rare diseases (RD) which when combined are estimated to affect up to 6-8 per cent of the population.[1] Rare diseases (RD) are a public health priority. In WA, they collectively affect up to 190,000 people, including 63,000 children.

Countries have different definitions of a rare disease. However, it has been proposed by the rare diseases community that Australia adopts the European Union consumer endorsed definition which refers to both prevalence and severity of burden.[2] This definition indicates that RD are "life-threatening or chronically debilitating diseases which are of such low prevalence (one in 2,000 people) that special combined efforts are needed to address them".[3,4]

Many RD onset during childhood and continue throughout life, although some do not become evident until adulthood. Around 80 per cent of RD have a known genetic association. Most cannot be prevented, are complex with multi-system dysfunction, disabling, incurable and have no effective treatment.[6,7] European studies show that 50 per cent of RD are associated with motor, sensory or intellectual impairment, 30 per cent of RD lead to an incapacity which reduces autonomy and 35 per cent of deaths that occur before the age of one year can be attributed to RD.[5]

Despite individual rarity, there are common healthcare needs expressed by those living with RD including achieving a timely diagnosis as a portal to best practice care. However, obtaining a timely accurate diagnosis is a particular challenge for individuals living with RD. In one study, including relatively well-known rare diseases like Marfan syndrome, 25 per cent of patients waited 5-30 years for a diagnosis; and in 40 per cent of cases, the initial diagnosis was wrong.[6]

Similar rates have recently been confirmed in Australia. A combination of awareness and systematic approaches, which pair new diagnostic tools with a clinician's expert knowledge, that are aligned with health system planning (see WA Rare Diseases Strategic Framework, 2015-2018),[7] is helping to address this diagnostic odyssey.

For previously diagnostically intractable cases, these approaches are obtaining a molecularly confirmed diagnosis in the order of ¼ of instances. Importantly, this still leaves a very significant proportion of undiagnosed individuals for which a coordinated approach is required.

CASE STUDY

Sibling 1

This Aboriginal girl was initially referred from, and seen in, a remote region at age two years and five months for genetic consultation for developmental delay and dysmorphia. Her parents were non-consanguineous, intellectually normal and had no known significant medical history. There was no known teratogen exposure.

Antenatal scans at 20 weeks gestation showed hepatomegaly and caesarean section was performed at 39 weeks due to fetal distress. Her birth weight was 3060g (median to -1SD) and there were no difficulties documented in the newborn period aside from a prominent startle-response. She crawled at 14 months and walked at two years of age. She had a few single words and no two-word phrases.

On examination, the proband was a hyperactive child with scaphocephaly, a normal hair-line with curly hair, frontal bossing, hypertelorism, down-slanting palpebral fissures, bi-temporal narrowing, hypotonic facies, an open mouth appearance, macrostomia, a prominent and long philtrum, flat nasal bridge, rocker-bottom heels, pes planus, broad feet, an ossifying anterior fontanelle, large ear lobes and a protuberant abdomen with an umbilicus that sat proud. Her height and weight were normal and her head circumference was greater than +3SD (53cm). She

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had one café-au-lait lesion and there was no axillary or inguinal freckling.

Abdominal ultrasound revealed renal asymmetry with the left kidney measuring at two standard deviations above the mean and the right kidney at the mean. A cerebral MRI showed mild prominence of the ventricular system, hypogenesis of the body and the splenium of the corpus callosum and generalised white matter loss, particularly in the peritrigonal regions. The mesencephalon, pons and medulla were small. The cerebellum was normal. Normal genetic investigations included subtelomeric FISH and G-banded karyotype. A provisional diagnosis of Sotos syndrome was considered and therefore NSD-1 analysis was performed and was normal.

On review at three years and three months of age, she had persistent developmental delay, particularly with speech. Her height and weight were normal and her head circumference was greater than +3SD.

On review at seven years of age, her height and weight were normal and her head circumference was 56.5 cm (> +3SD). An echocardiogram showed a tiny aortic sinus to right atrium fistula.

Sibling 2

This boy, a maternally related half-sibling, was initially seen for genetic consultation at three weeks of age. He was born at 36 weeks with a weight of 3855g (> +3SD), a head circumference of 37cm (> +3SD) and a length of 51cm (-1 to -2SD). He had neonatal hypotonia, a similar pattern of facial dysmorphism to his sister, a relatively narrow thorax, an umbilical hernia and redundant back and neck skin.

A diagnosis of a RASopathy, most particularly Costello syndrome, was considered. The following genetic investigations were normal: PTPN-11, SOS-1, KRAS, HRAS and RAF- 1 testing; and given the syndromic intellectual disability chromosomal microarray was also performed and was normal. A cranial ultrasound was normal and a renal ultrasound revealed normal renal sizes and no asymmetry.

At six months of age, his head circumference was 49.6cm (> +3SD) and he had a normal height and weight. A nasogastric tube was in-situ.

Other examination findings included mild facial coarsening, a distended abdomen and, even allowing for this, his thorax looked small. Other findings included lax skin, particularly over the thighs; excessive plantar wrinkling; deep palmar creases; excessive knuckle creases; hepatomegaly; and macro-orchidism.

He subsequently suffered from recurrent viral respiratory tract infections with multiple hospital admissions, including an adenovirus infection. He was found to have isolated IgA deficiency.

At the request of his Paediatrician who raised the possibility of NF-1, he was reviewed at two years of age. He remained macrocephalic and had a very sociable personality, absent speech, soft skin, and had multiple café-au-lait lesions. He had no Lisch nodules; NF1 testing was normal.

A cranial MRI at two and a half years of age showed megalencephaly, perisylvian polymicrogyria, mild prominence of the lateral ventricles, moderate hypogenesis of the corpus callosum, three small areas of heterotopic grey matter within the right frontal lobe. Given these findings, the possibility of a megalencephaly-associated syndrome was considered.

At three years and one month of age, his height and weight were normal and his head circumference was 57.3 cm (> +3SD). He was not crawling and had 3-4 words.

Sibling 3

This boy was a maternally related half-sibling who was seen at two years and two months of age. He had been in foster care for 12 months and upon receipt into care had a persistent head lag, could roll from front to back and couldn't sit. He could crawl and pull himself up onto the furniture. A Griffith assessment at 25 months showed an age equivalence of nine months. He had no words with meaning and could eat "pea-sized" foods without choking.

On examination, he was a very active child with a head circumference of 54.5 cm (> +3SD) and a normal height and weight. His facial and connective tissue findings were similar to his siblings. A review of infantile photos demonstrated a small thorax.

Examination of the children's mother was unremarkable aside from one café-au-lait lesion.

Given the phenotype overlapped the RASopathies and (hemi) megalencephaly-associated syndromes, whole exome analysis was bioinformatically targeted to analyse for variants in (19) genes in the RAS-MAPK and interrelated (hemi) megalencephaly-associated pathways. The gene variants were then filtered according to a number of criteria including allele frequency in available, largely Caucasian, reference datasets. A single variant was identified. Lines of biochemical evidence and in-silico analysis supported that this variant was possibly disease causing (pathogenic). Using Sanger sequencing,

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we confirmed the presence of the variant in all three affected individuals and its absence in their unaffected mother.

Despite this promising supportive information, given that variants in this gene had not been previously associated with familial disease, and the absence of an Aboriginal Australian genetic reference range at against which to interpret the findings, we did not have the level of certainty about the nature of the known variant that is required for clinical care. We therefore sought further confirmatory studies.

In short, after a prolonged search, we were able to obtain functional cellular studies that showed a gain-of-function (overactivity) effect of this mutation. Importantly, cellular correction was demonstrated with the administration of a drug know to inhibit this genes effect (an mTOR inhibitor; Rapamycin). This provided a novel therapeutic possibility for this family.

ILLUSTRATIVE POINTS FROM THIS FAMILY
THE POWER OF A DIAGNOSIS

BENEFITS	THIS FAMILY	COMMENTS
Certainty – psychological relief	yes	The power of knowing the cause of the condition and improved prognostication.
Reduced isolation	yes	A further (non-Aboriginal) child has been identified with an mTOR mutation, offering the possibility of connection for shared experience.
Reduce unnecessary investigations	yes	No further need for investigations, which may be invasive and/or costly.
Access to improved or best practice medical care, including reducing inappropriate management	yes	Targeted follow-up and surveillance by what is known from related disorders. Possibility of drug repurposing*.
Clarify recurrence risk	yes	Whilst there is clearly a risk of recurrence for this mother, risk of recurrence for other family members is essentially zero.
Provide additional reproductive options	yes	A molecularly confirmed genetic diagnosis provides options for prenatal or pre-implantation genetic diagnosis.
Access to social and educational Services	unclear	No specific access in this instance, however it is the case for selected other rare disorders.

*drug repurposing: using a given drug for a new indication (disease).

THE IMPORTANCE OF PHENOTYPE

Medical phenotyping – detailed clinical assessment (history, including family history; examination and investigations) remains the cornerstone of medical diagnostics and care provision.[8] This is increasingly important in an era of genomic investigations.

Recently, high throughput genetic sequencing (next-generation sequencing) has allowed the parallel sequencing of a large number of genes known to be associated with a particular phenotype (clinical presentation). Depending on how this is applied, it can detect none, one, a handful, to more than 100 potentially disease-causing variants per person. It can therefore be a very substantial challenge to determine **if** one or none of these variants is related to the condition being investigated. Detailed and precise phenotyping of the proband, and often family members, is crucial for test interpretation by reducing pre-test probability and otherwise informing test analysis. The clinical phenotype in this family was critical to the test design and result interpretation.

EQUITABLE GENETIC HEALTHCARE

Rare genetic variants are disproportionately important in terms of complex disease risk and pharmacogenomics and some are the cause of monogenic disease. Rare variations tend to be population specific. Accordingly, reference data from historically marginalised populations are needed to separate real from spurious finding.[9] Advances in genetic testing that are being increasingly applied for clinical care therefore require engagement of Indigenous communities, so that normal genetic variation in these populations can be ascertained.[10] Failure to prioritise these studies will perpetuate health inequity. In this family, the lack of Aboriginal genomic reference data caused a diagnostic delay (an additional 18 months). This is of notable importance here given the possibility of drug repurposing.

RED FLAGS FOR GENETIC AND RARE DISEASE

Whelan et al[11] created the mnemonic "Family GENES" as a red flag for genetic conditions. This can be applied to prompt the question – "Is this a rare disease?" ■

FAMILY GENES

Family history: multiple affected siblings or individuals in multiple generations. Remember that lack of a family history does NOT rule out genetic causes

G: group of congenital anomalies. Common anatomic variations are, well, common; but two or more anomalies are much more likely to indicate the presence of a syndrome with genetic implications.

E: extreme or **exceptional presentation of common conditions.** Early onset cardiovascular disease, cancer, or renal failure. Unusually severe reaction to infectious or metabolic stress. Recurrent miscarriage. Bilateral primary cancers in paired organs, multiple primary cancers of different tissues.

N: neurodevelopmental delay or degeneration. Developmental delay in the paediatric age group carries a very high risk for genetic disorders. Developmental regression in children or early onset dementia in adults should similarly raise suspicion for genetic etiologies.

E: extreme or exceptional pathology. Unusual tissue histology, such as pheochromoctyoma, acoustic neuroma, medullary thyroid cancer, multiple colon polyps, plexiform neurofibromas, multiple exostoses, most paediatric malignancies.

S: surprising laboratory values. Transferrin saturation of 65 per cent, potassium of 5.5 mmol/L, and sodium of 128 mmol/L in an infant; cholesterol of >500 mg/dL and unconjugated bilirubin of 2.2 mg/dL in an otherwise healthy 25-year-old; phosphate of 2 mg/dL and glucose of 35 mg/dL in a six-month-old child.

All of these flags were present in the family in the case study.

References available on request.