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. 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. 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.”

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. Studies show that 50 per cent of RD are associated with motor, sensory or intellectual impairment, 50 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.

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. 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), is helping to address this diagnostic odyssey.

For previously diagnostically intractable cases, these approaches are obtaining a molecularly confirmed diagnosis in the order of 1⁄3 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 teratogenic 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 3000g (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-sloping palpebral fissures, a temporolateral narrowing, hypertonic face, an open mouth appearance, macrostomia, a prominent and long philtrum, flat nasal bridge, rocker-bottom heels, pes planus, broad feet, an osifying anterior fontanelle, large ear lobes and a protuberant abdomen with an umbilical hernia that sat proud. Her height and weight were normal and her head circumference was greater than +3SD (53cm).

**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 33cm (> +3SD) and a length of 51cm (-1 to -2SD). He had neonatal hypotonia, a similar open mouth appearance, macrostomia, a prominent and long philtrum, flat nasal bridge, rocker-bottom heels, pes planus, broad feet, an osifying anterior fontanelle, large ear lobes and a protuberant abdomen with 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: PTPN11, SOS1, KRAS, HRAS and RAF 1 testing; and given the syndrome’s 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 cafe-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 hypoplasia 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 57.3cm (> +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 54.6 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 cafe-au-lait lesion. Given the phenotype overlapped the RASopathies and (ben) megalencephaly-associated syndromes, whole exome analysis was bioinformatically targeted to analyse for variants in (19) genes in the RAS-MAPK and interrelated (beni) 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 possible disease causing (pathogenic). Using Sanger sequencing.
A Diagnostic Odyssey – Red Flags in the Red Sand
Dr Gareth Baynam

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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 (oncogenicity) effect of this mutation. Importantly, cellular correction was demonstrated with the administration of a drug known to inhibit this gene effect (an mTOR inhibitor; Rapamycin). This provided a novel therapeutic possibility for this family.

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. 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 one, or 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. 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. 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 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?”

ILLUSTRATIVE POINTS FROM THIS FAMILY

THE POWER OF A DIAGNOSIS

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