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NEXT GENERATION SEQUENCING FOR DIAGNOSIS OF RETT SYNDROME AND RELATED DISORDERS

Rett syndrome encompasses a spectrum of neurological disorders. Females are predominantly affected and presentation ranges from classic Rett syndrome to variant (atypical) Rett syndrome or milder learning disabilities. Classic Rett syndrome is most often due to a pathogenic variant in the *MECP2* gene located on the X chromosome. It is a severe, progressive disorder, usually presenting after apparent 'normal' development in the first 6 to 18 months of life. After a developmental plateau, there is a rapid regression of skills, stereotypic movements, seizures, acquired microcephaly, ataxia and dysautonomic features. Presentation of males with *MECP2*-related disorders ranges from a severe neonatal encephalopathy to pyramidal signs, parkinsonism and macroorchidism (PPM-X) to severe intellectual disability.

Pathogenic variants in the *MECP2* gene are the main cause of Rett syndrome. However, approximately 5% of patients with classic Rett and 25% of patients with atypical Rett syndrome do not have a causative *MECP2* variant. Variants in other genes are now known to present with a Rett-like phenotype, often clinically indistinguishable from Rett syndrome. For example, variants in the *CDKL5* gene are described in patients with an early seizure onset form of Rett syndrome, and variants in the *FOXG1* gene cause a 'congenital' presentation of Rett syndrome. Clinical features of patients with *MEF2C* pathogenic variants overlap with features of Rett syndrome, and pathogenic variants in the *UBE3A* gene are the cause of about 11% of Angelman syndrome cases, the features of which also overlap with those of Rett syndrome.

Historically, analysis of the *MECP2* gene has been performed using only targeted Sanger sequencing focusing initially on the 'hotspot' regions in exons where the majority of variants are found. Next generation sequencing (NGS) technology is now able to comprehensively sequence the *MECP2* gene together with other genes responsible for this genetically heterogeneous condition. This is possible at a similar cost to the previous, less comprehensive approach. Ampath has recently implemented an in-house designed and validated NGS panel, run on the Ion Torrent platform. This panel is combined with a commercial MLPA kit, allowing for the detection of deletions/duplications in order to investigate and diagnose these conditions more effectively (summarised in Table 1).

TABLE 1: GENES AND METHODS

Gene	Inheritance	Clinical presentation	NGS	MLPA
MECP2	XLD	Classic Rett syndrome	Exons 1-4; accounts for 90% of variants	Exons 1-4; accounts for 10% of variants
CDKL5	XLD	Atypical Rett syndrome	Exons 2-18	Exons 3, 6, 9 and 10
FOXG1	AD	Atypical Rett syndrome	Exon 1	N/A
MEF2C	AD	Atypical Rett syndrome	Exons 2-11	N/A
UBE3A	AD	Angelman syndrome	Exons 3-13	*

MLPA: Multiplex Ligation-dependent Probe Amplification; XLD: X-linked dominant; AD: Autosomal dominant; N/A: Not applicable

* Variants in *UBE3A* only account for about 11% of Angelman syndrome cases. First-line testing for Angelman syndrome is requested separately using a methylation sensitive MLPA technique.

TABLE 2: TEST INFORMATION

Genes included	***MECP2, CDKL5, FOXG1, MEF2C*** and ***UBE3A***
Specimen type	Peripheral blood sample (EDTA)
Test mnemonic	RETT
Cash price*	R5 224.00
Turnaround time**	6 weeks

* This price is valid until 31 December 2024.

** Once received at the National Reference Laboratory.

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REFERENCES AVAILABLE ON REQUEST