Technical Note:

Exome limitations observed in clinical practice

This technical note documents exome testing limitations that we have observed first-hand in the Variantyx lab. In each case, a patient was impacted by delayed diagnosis.

Clinicians in a wide variety of medical settings use genetic testing with the aim of identifying the etiology of a patient's symptoms and bringing a quick end to the diagnostic odyssey. Including newborns and children in time-sensitive NICU and PICU environments for whom a delay in diagnosis due to iterative, prolonged testing can have life-threatening consequences.

Exome testing has become a key diagnostic tool. This is due to its broad gene coverage and resulting ability to outperform targeted, panel-based tests when compared in published clinical studies. Whole genome testing has been investigated as an alternative to exome testing, with positive results, yet clinicians are often hesitant to switch to the newer technology.

As a laboratory that performs testing based exclusively on a whole genome platform, we have an up-close view of the comparative limitations of exome testing. Frequently we receive samples from patients who have had exome testing with negative or non-diagnostic results. Many of these patients subsequently receive a diagnosis as a result of our testing.

We see three recurrent exome testing limitations that explain the difference in results: missed variants due to expected non-coverage, unexpected non-coverage and known limitations of the technology.

Expected non-coverage
Exome testing offers broad gene coverage, but not all genes are considered. Non-protein coding genes are frequently excluded or absent and mitochondrial genes are not always included by default. mtDNA coverage oftentimes requires a separate test order. By focusing on exonic regions, deep intronic variants are routinely missed by exome testing, even when clinically relevant.

Unexpected non-coverage
Exome testing is commonly expected to provide complete coverage of exons as well as immediately flanking intronic sequences important for regulation and splicing: +/-10-20bp from the exon/intron junction, depending upon the provider. In clinical practice numerous SNVs and indels are missed, apparently due to poor probe coverage and/or the sequence environment.

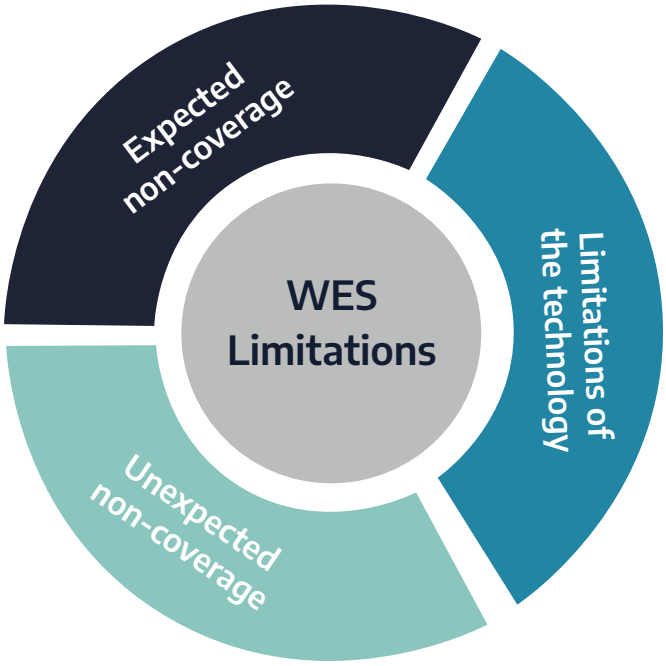
Known limitations of exome technology
Exome testing has known limitations including detection of small CNVs (≤3 exons in size), complex structural variants (inversions, MEIs, aneuploidy, ROH/UPD) and short tandem repeat expansions.

See the opposite page for examples of all three limitations observed in clinical practice, or explore our case studies directly.

Genomic Unity® whole genome platform case studies

Mitochondrial, deep intronic and non-protein coding variants

Type	Gene	Variant(s)	Diagnosis
Mitochondrial	*MT-ATP6*	m.8993T>G	Mitochondrial disease
Deep intronic	*DMD*	c.2949+964G>A	Duchenne muscular dystrophy
Deep intronic	*SNRPB*	g.2447952C>G	Cerebrocostomandibular syndrome
Non-protein	*RNU7-1*	n.28C>T; n.40_47delCTGGCTTT	Aicardi-Goutieres syndrome 9
Non-protein	*RNU4ATAC*	n.122G>A; n.28T>C	RNU4ATAC-related disorders
Non-protein	*RNU4ATAC*	n.16G>A; n.16G>A	RNU4ATAC-related disorders



Small CNVs

Type	Gene	Variant(s)	Diagnosis
<1 exon CNV	*MECP2*	139bp del	Rett syndrome
1 exon CNV	*TBCK*	~7kb del	TBCK-related encephalopathy
1 exon CNV	*ARID1B*	~2.4kb del	Coffin-Siris syndrome
1 exon CNV	*EFTUD*	~2.3kb del	Mandibulofacial dysostosis with microcephaly
1 exon CNV	*PEX1*	~4.86kb del	Zellweger syndrome
1 exon CNV	*FITM2*	~8.72kb del	Siddiqi syndrome
1 exon CNV	*TRIO*	~3kb del	TRIO-related neurodevelopmental disorder
2 exon CNV	*MYH2*	~1.9kb del	MYH2-related myopathy

Large structural variants and STRs

Type	Gene	Variant(s)	Diagnosis
Inversion	*SATB2*	2.9Mb inv	Glass syndrome
Inversion	*SOX5*	9.3Mb inv	Lamb-Shaffer syndrome
MEI	*VPS13A*	AluY ins	Chorea-acanthocytosis
Aneuploidy		47,XY,+21[20]/45,X[5]	Turner syndrome
UPD		~86Mb upd	Temple syndrome
STR	*FMR1*	Full expansion	Fragile X syndrome

Exonic and near intronic SNVs and indels

Type	Gene	Variant(s)	Diagnosis
Exonic indel	*SYNGAP1*	c.1167_1168delAG	SYNGAP1-related non-syndromic intellectual disability
Exonic SNV	*ZMYND11*	c.1246G>A	ZMYND11-related syndromic intellectual disability
Exonic indel; Exonic indel	*BBS10*	c.271dupT; c.9_15delTTCTATGinsGC	Bardet-Biedl syndrome
Near intronic indel	*LAMP2*	c.864+3_864+6delGAGT	Danon disease
Near intronic SNV, Exonic indel	*SBDS*	c.258+2T>C; c.183_184delTAinsCT	Shwachman-Diamond syndrome
Near intronic SNV, 1 exon del	*TBCK*	c.1220+5G>A; ~7kb deletion	TBCK-related encephalopathy

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