P599: Beyond Single Nucleotide Variants and Copy Number Variations: Spinal Muscular Atrophy and Repeat Expansion Disorders Screening by Whole Genome Sequencing

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1 Background

- Whole genome sequencing (WGS) has been utilized as the first tier, cost-effective genetic testing for both clinical settings and healthy screening supported by unbiased coverage to identify single nucleotide variants (SNV) and large copy number variants (CNV). However, due to the complex disease-causing mechanisms, chromosome structures, and genetic components, the detection of disorders caused by homologous genes such as spinal muscular atrophy (SMA) and repeat expansions has been challenging.
- (SMA) is a progressive motor neuron disease affecting approximately 1 in 10,000 live births. Approximately 95% of individuals with SMA have a homozygous deletion of the *SMN1* exon 7. *SMN1* and *SMN2* encoding survival motor neuron (SMN) proteins lie within the telomeric and centromeric halves on chromosome 5 and share more than 99% nucleotide identity.
- Repeat expansion disorders are a group of clinically and genetically heterogeneous diseases that are caused by expansions of short tandem repeats (STR). Thus far polymerase chain reaction (PCR) and /or Southern blot are the gold standard approaches for molecular diagnosis of repeat expansion disorders.
- Identification of SMA and STR-associated diseases has not been universally implemented in WGS.

2 Methods

- We integrated SMA and STR expansion screening into our WGS platform by using bioinformatics tools based on published literature (PMID: 28125085, 32092542, 28887402).
- For SMA analysis, exon 7 in both *SMN1* and *SMN2* is screened by WGS. Positive screening is confirmed by multiplex ligation-dependent probe amplification (MLPA). SMA carrier analysis cannot distinguish “1+1” from “2+0” (silent carriers) of the *SMN1* gene.
- For repeat expansion analysis, a total of 31 repeat expansion disorders were evaluated from the WGS data. STR screening results were defined into three categories described in ACMG poster eP355 (03-2022).

3 Results

3.1 A total of 1337 clinical WGS cases including singleton and trio were analyzed. We detected 4 SMA patients, 25 SMA carriers, and 8 cases with potential or confirmed STR disorders (Table 1).

Table 1: The number of detected SMA and STR cases by WGS

Patient Cohort	No. of Cases
Total	1337
SMA	4
SMA Carrier	25
STR	8

3.2 The SMA cohort with 0 copies of *SMN1* exon 7 (Table 2, d: days; mo: months; yo: years old):

- **The cohort included 2 children and 2 adults, 3 females and 1 male.**
- **None of these cases were referred for WGS testing due to a clinical suspicion of SMA.**
- **None of these cases were reported as newborn screening (NBS) positive for SMA.**
- **Case 1 had a variant of uncertain significance in *FLT4* associated with lymphatic malformation and congenital heart defect.**
- **Case 2 had neuromuscular and neuropathy panels testing previously and the results were non-diagnostic.**
- **Case 5 was detected through WGS healthy screening.**

Table 2: The demographic information, clinical presentation and copy number of SMN2 in the SMA cohort.

Patient Cohort	Case#/ Sex	Age at testing	Reported clinical features	Copy number of *SMN2*
Pediatric	Case 1/ Female	12 d	Cystic hygroma, hydrops fatalis, anasarca, lymphatic malformation, hypoplastic transverse arch, muscular ventricular septal defect, patent ductus arteriosus	1
	Case 2/ Female	5 yo	Limb girdle weakness, positive Gowers sign, idiopathic gait dysfunction, femoral anteversion, hypermobility, proximal muscle weakness	4
Adult	Case 3/ Male	18 yo	Muscular dystrophy, limb-girdle muscular dystrophy, abnormal creatine kinase, muscular hypotonia	4
	Case 4/ Female	56 yo	Congenital motor neuropathy, sensory neuropathy	4
Healthy screening	Case 5/ Male	6 mo	NA	4

3.3 The characteristics of the potential or confirmed repeat expansion cases associated with the patient’s phenotype:

- **The cohort included 5 children (3 females and 2 males), and 3 adult males.**
- **The identified STR genes are *PHOX2B* (3 cases), *ATXN2, PABPN1, ATXN7, DMPK* and *ZIC2* genes.**
- **The repeat expansion has been confirmed by direct sequencing analysis (case 5 in Table 3), tool-assisted sequencing analysis (case 3 in Table 3, PMID 37838930), or repeat-primed PCR assay (cases 1 and 4 in Table 3).**

Table 3: The demographic information, clinical presentation and repeat expansion disorders identified in example cases by WGS.

Patient Cohort	Case # / Sex	Age at testing	Main phenotypes	STR Gene	STR alleles	Confirmed expansion
Pediatrics	Case 1/ Female	2 yo	Cone-rod dystrophy, cerebellar atrophy, leukodystrophy, global developmental delay, developmental regression, intellectual disability, ataxia, hypotonia, seizure, dysphagia	*ATXN7*	10-10/56-80	10/>125
	Case 2/ Male	5 d	Muscular hypotonia and wasting, abnormal external genitalia, cryptorchidism, right single palmar crease, preterm birth	*DMPK*	13-13/74-127	Pending
	Case 3/ Feale	5 mo	Apnea, neonatal respiratory failure, hypoventilation, hypoxemia	*PHOX2B*	20-20/33-33	20/33
Adult	Case 4/ Male	42 yo	Generalized central nervous system atrophy, tremor, ataxic gait, dysmetria, slowed and dysarthric speech	*ATXN2*	23-23/44-44	23/44
	Case 5/ Male	18 yo	Dysphagia, deambulation, anarthria, myoclonic epilepsy, axonal polyneuropathy, cognitive decline	*PABPN1*	10-10/17-17	10/17

Table 4: The general information of the five repeat expansion disorders demonstrated in Table 3.

Disease category	Gene	Inheritance	Disorder (Curated PMID)	Nucleotide repeat	Repeat location	Normal repeat number	Premutation	Reduced-penetrance pathogenic repeat	Full-penetrance pathogenic repeat
Neurodegenerative	*ATXN2*	AD	Spinocerebellar ataxia type 2 (20301452)	CAG	Exon1	<=31	-	33-34	37
	ATXN7	AD	Spinocerebellar ataxia type 7 (20301433)	CAG	Exon1	4-27	26-33	34-36	37
Neuromuscular	*DMPK*	AD	Myotonic dystrophy type 1 (20301344; 32851192)	CTG	3'UTR	5-34	35-49	-	50
	PABPN1	AD	Oculopharyngeal muscular dystrophy (20301305)	GCN	Exon1	<=10	-	-	11-18
Respiratory	*PHOX2B*	AD	Congenital central hypoventilation syndrome (20301600)	GCN	Exon3	<=20	-	-	25-33

4 Summary

- ✓ **WGS screening facilitated the identification of SMA or STR disorders, especially for those with atypical clinical manifestations and late age of onset.**
- ✓ **WGS screening detected carriers of SMA to guide reproductive and family planning.**
- ✓ **The utilization of short-read WGS to screen SMA and STR disorders will increase the clinical diagnostic yield, benefit carrier screening, and allow for accurate genetic counseling and medical management.**