Expanding Spinal Muscular Atrophy Diagnosis Through Multi-Gene Panel Testing

Christopher Tan[1], Jody Westbrook[1], Rebecca Truty[1], Tom Winder[1]
[1]Invitae, San Francisco, CA

BACKGROUND

Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by the loss of motor neurons within the spinal cord, which results in progressive muscle weakness and atrophy. The age of onset, clinical presentation, prognosis and life expectancy display marked inter- and intra-familial variability making diagnosis challenging due to the broad differential in those with a milder presentation.

Adding to this complexity is the molecular genetics of SMA, caused by loss of *SMN1* and mitigated by *SMN2* copy number, which is highly variable amongst individuals, and differs from *SMN1* coding region at a single nucleotide position. By integrating *SMN1* and *SMN2* into next-generation sequencing (NGS) analysis, we sought to determine whether its inclusion in a broad multi-gene neuromuscular panel would detect undiagnosed SMA patients.

METHODS

- We performed NGS analysis of up to 122 genes, including *SMN1* and *SMN2*, in 3204 patient samples as part of a comprehensive neuromuscular multi-gene panel.
- Simultaneous single nucleotide and copy number variant analysis was performed on the included genes.
- *SMN1* and *SMN2* analyses were performed using a validated bioinformatic approach and high-depth NGS derived from both *SMN1* and *SMN2*.
- Combined reads were aligned to the *SMN1* reference sequence, and combined *SMN1/2* copy number was determined using an NGS-based copy number variant detection algorithm called CNVitae.
- *SMN1*- and *SMN2*-specific exon 7 copy number was resolved by analyzing the ratio of the c.840C>T gene determining variant in exon 7 (Figure 1).
- All pathogenic results were confirmed by an orthogonal method.

CONCLUSIONS

The analytical platform described here permits SMA analysis to be included in a broader phenotypic panel with the intent to diagnose patients with unclear or broad phenotypes that may not warrant single gene testing. The molecular diagnosis of SMA utilizing NGS is a further improvement to providing a more comprehensive and simultaneous analysis of neuromuscular conditions with a single assay.

RESULTS

- Homozygous loss of *SMN1* exon 7 was detected in 22 patients, establishing a diagnosis of SMA. (Table 1). *SMN2* copy number ranged from 2 (n=5) in infants, and up to 3 (n=9) and 4 (n=8) in the remaining individuals (Table 2)
- Heterozygous loss of *SMN1* exon 7 was detected in 78 patients. Of those, 9 had an additional indeterminate variant in either *SMN1* or *SMN2* that could not be disambiguated with this assay (Table 3).
- Of the remaining SMA carriers identified, 26 patients had pathogenic variants in other genes detected (Table 4)

Table 1. Patients with homozygous loss of *SMN1* exon 7

	Patients
Total	22
Age at exam in years: mean, median [range]	16, 6 [0.2-68 years]
Reason for referral:	
Symptoms highly suspicious of SMA	4/22
Hypotonia	3/22
Muscle weakness	6/22
Motor delay	2/22
Muscular dystrophy	3/22
Not provided	7/22

Table 2. *SMN2* copy number for patients with homozygous loss of *SMN1* exon 7

Ages	*SMN2* copy number			
	1	2	3	4
Birth – 2 years	0	5	1	0
3 – 8 years	0	0	4	3
9- 18 years	0	0	1	1
18 and above	0	0	2	5

Figure 1.

NGS-based method for detecting *SMN1* and *SMN2* copy number variants

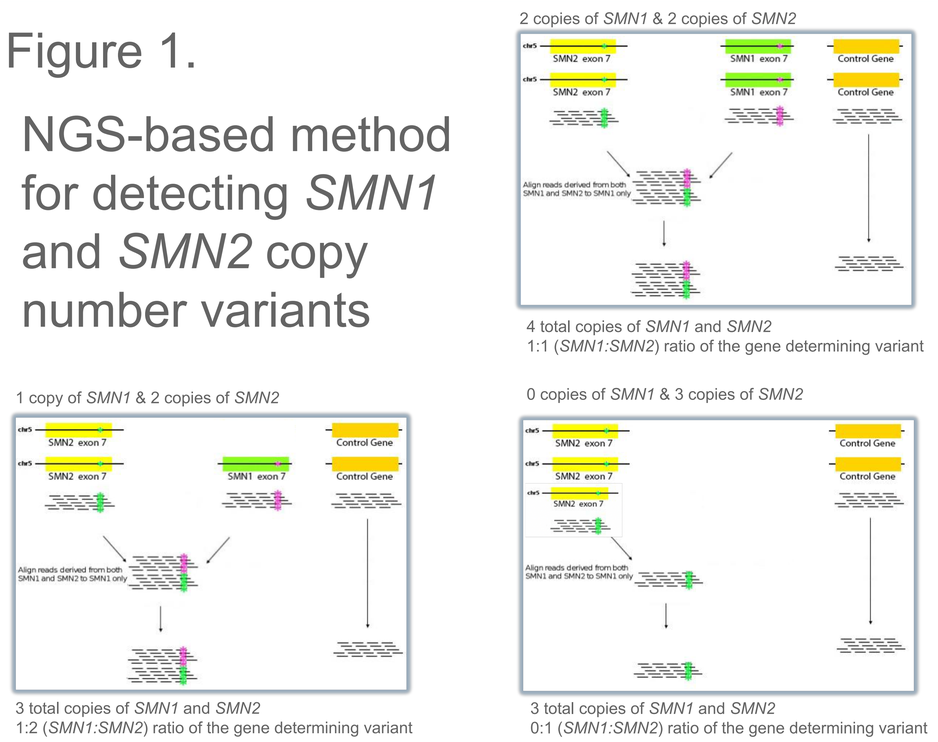

Table 3. Indeterminate variants in patients with heterozygous loss of *SMN1* exon 7

SMN1 or *SMN2* variant
Partial Deletion (Exon 6)
c.815A>G (p.Tyr272Cys)
c.824G>T (p.Gly275Val)
Deletion (Exons 1 - 4)
Deletion (Exon 1)
c.5C>G (p.Ala2Gly)
c.283G>C (p.Gly95Arg)
Deletion (Exons 1- 6)
c.475-2A>T (Splice acceptor)

Traditionally, the diagnosis of SMA is performed as a standalone test by multiplex ligation-dependent probe amplification or quantitative PCR to identify loss of *SMN1*. By performing testing by NGS, we were able to detect 9 indeterminate variants in individuals with heterozygous loss of *SMN1* exon 7. Resolution of the location of the indeterminate variants may support a diagnosis of SMA in those individuals.

Table 4: Other Pathogenic variants identified in patients with heterozygous loss of *SMN1* exon 7

Definitive Diagnosis[1]			
CAPN3	NM_000070.2:c.985G>A (p.Gly329Arg), het	*GAA*	c.-32-13T>G (Intronic), het
	NM_000070.2:c.1469G>A (p.Arg490Gln), het		c.2281delinsAT (p.Ala761Ilefs*35), het
CLCN1	NM_000083.2:c.1129C>T (p.Arg377*), het	*LMNA*	NM_170707.3:c.1072G>A (p.Glu358Lys), het
	NM_000083.2:c.751delT (p.Ser251Leufs*17), het	*RYR1*	NM_000540.2:c.14693T>C (p.Ile4898Thr), het
DNM2	NM_001005360.2:c.1393C>T (p.Arg465Trp), het	*RYR1*	NM_000540.2:c.7354C>T (p.Arg2452Trp), het
FHL1	NM_001449.4:c.358_361dupACCG (p.Val121Aspfs*11), hemi	*SGCG*	NM_000231.2:Deletion (Exons 1-2), homo
FKRP	NM_024301.4:c.826C>A (p.Leu276Ile), homo	*TRIM32*	NM_012210.3: Deletion (Entire coding sequence), homo
Carrier[2]			
ACTA1	NM_001100.3:c.782A>T (p.Glu261Val), het	*GAA*	c.-32-13T>G (Intronic), het
AGRN	NM_198576.3:c.902_912delGCGCCTGCGCC (p.Arg301Profs*14), het		NM_000152.3:c.1441T>C (p.Trp481Arg), het
ALMS1	NM_015120.4:c.873delC (p.Ser291Argfs*12), het		NM_000152.3:c.2242dupG (p.Glu748Glyfs*48), het
ALS2	NM_020919.3:c.1233T>G (p.Tyr411*), het	*NEB*	NM_001271208.1:c.19944G>A (Silent), het
CAPN3	NM_000070.2:c.2393C>A (p.Ala798Glu), het		NM_001271208.1:c.24072_24075del (p.Pro8025Serfs*154), het
CLCN1	Partial Deletion (Exons 1-3), het	*POMGNT1*	NM_017739.3:c.643C>T (p.Arg215*), het
CPT2	NM_000098.2:c.338C>T (p.Ser113Leu), het	*RYR1*	NM_000540.2:c.11763C>A (p.Tyr3921*), het
DPAGT1	NM_001382.3:c.26dupT (p.Met9Ilefs*80), het	*TOR1AIP1*	Partial Deletion (Exon 8), het
FKBP14	NM_017946.3:c.362dupC (p.Glu122Argfs*7), het		

[1]an observation of heterozygous LP/P variant in a dominant or XL disorder, or two LP/P variants or a homozygous LP/P variant for a recessive disorder.
[2]an observation of a heterozygous LP/P variant in a recessive disorder

Disclosures: Authors are stockholders and employees of Invitae.