Reducing The Time To Diagnosis For Spinal Muscular Atrophy

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BACKGROUND

- SMA is the most common neurodegenerative disease in childhood with an incidence of 1 in 6,000 to 1 in 10,000. SMA is caused by deleterious changes in the *SMN1* gene, with a deletion of exon 7 being the most common pathogenic event. Homozygous deletion of exon 7 can be found in approximately 95% of SMA cases, whereas the other 5% are compound heterozygous of this deletion.

- **Fragment analysis using the AmplideX® PCR/CE *SMN1/2* Plus Kit.** The assay is based on PCR and capillary electrophoresis. In addition to *SMN1/2* copy number determination, the assay also detect the presence/absence of gene duplication maskers and modifier c.*3+80T>G, c.*211_*212del, and c.859G>C.

- **Identification of SMA positive (*SMN1*=0) and *SMN1* carrier statues using WGS data.** A bioinformatic workflow was developed and validated for the *SMN1* copy number determination through uniquely mapped reads on exon 7 of *SMN1* gene using the WGS data. Median read depth of *SMN1/SMN2* exon 7 for WGS samples from previous runs are computed and used for normalization.

Validation for the Fragment Analysis Assay

A total of 20 unique samples with known *SMN1/2* copy numbers and /or known variants (15 coriell samples and 5 whole blood clinical samples) selected initially for validation. Two were excluded due to lack of enough gDNA for further testing. All the test results are as expected.

Results summary of analysis of Coriell and patient samples with known outcomes.

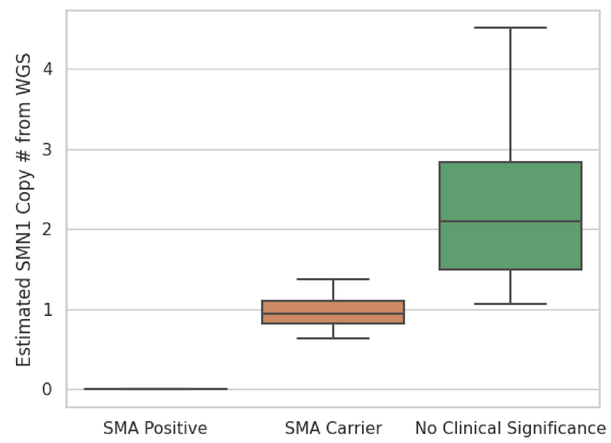
Identifier	*SMN1* Copy Number	SMN2 Copy Number	c.*3+80 T>G	c.*211_*21 2del	c.859G >C	Hybrid Peak	As expected?	Analysis Date	Plate Barcode
SMAVAL0101	0	2	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0102	0	3	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0103	1	≥4	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0104	1	1	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0105	0	3	No	No	No		Yes	6/24/2020	C9H11A9F
SMAVAL0106	0	2	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0107	2	0	No	No	No		Yes	6/23/2020	C9H11A9H
SMAVAL0108	3	0	Yes	Yes	No		Yes	6/25/2020	C9H11A9E
SMAVAL0109[1]	≥4	0	Yes	Yes	No	SMN1	Yes	6/23/2020	C9H11A9H
SMAVAL0110	≥4	1	Yes	Yes	No		Yes	6/19/2020	C9H11A79
SMAVAL0111	2	2	No	No	Yes		Yes	6/19/2020	C9H11A79
SMAVAL0112	0	3	No	No	No		Yes	6/24/2020	C9H11A9F
SMAVAL0113	0	3	No	No	No		Yes	6/24/2020	C9H11A9F
SMAVAL0114	1	2	No	No	No		Yes	6/19/2020	C9H11A79
SMAVAL0115	2	2	No	No	No	SMN2	Yes	6/24/2020	C9H11A9F
SMAVAL0116	QNS								
SMAVAL0117	1	3	No	No	No		Yes	6/25/2020	C9H11A9E
SMAVAL0118	2	2	No	No	No		Yes	6/24/2020	C9H11A9F
SMAVAL0119	QNS								
SMAVAL0120	2	0	No	No	No		Yes	6/24/2020	C9H11A9F

[1] 2 copies of *SMN1* and 2 copies of *SMN1* hybrid

Identification of SMA (*SMN1*=0) and *SMN1* carrier statues using WGS data

1) Determination of reference range for *SMN1* copy number

A total of 76 samples were analyzed using the bioinformatic tool, *SMN1* copy numbers were confirmed by MLPA or fragment analysis, and reference ranges were determined for SMA positive (*SMN1*=0), SMA carrier (*SMN1*=1), and no clinical significance (*SMN1*>=2).



2) Identify SMA patients (*SMN1*=0) using WGS data

Three DBS samples were used. The bioinformatic tool identified all 3 as *SMN1*=0.

Index	ACCESSION	Sex	Bait	Sample type	SMN1_EST_COPY	SMN2_EST_COPY	Orthogonal Method	SMN1_REF_COPY	SMN2_REF_COPY
1	V2020048296	F	WGS	Dried Blood Spots	0	2.39	MLPA	0	2
2	V2020074696	M	WGS	Dried Blood Spots	0	3.11	MLPA	0	3
3	V2020141248	F	WGS	Dried Blood Spots	0	2.31	MLPA	0	2

3) Identify carrier statues of *SMN1* using WGS data

WGS samples with estimated *SMN1* copy # within the reference range for SMA carrier were reflexed to the MLPA confirmation test. Here are examples of several clinical samples.

Sample ID	Estimated *SMN1* Copy# by WGS	MLPA Confirmed Copy #
WGS #1	1.13	1
WGS #2	1.04	1
WGS #3	0.83	1
WGS #4	0.71	1
WGS #5	1.16	2
WGS #6	0.86	1
WGS #7	1.33	1
WGS #8	1.09	1
WGS #9	1.33	2

CONCLUSION

- Currently, this laboratory performs qPCR assay for population-based newborn screening for SMA, bioinformatic analysis of WGS data for identification of SMA patients (*SMN1*=0) and *SMN1* carrier statues, fragment analysis and MLPA for confirmation and diagnostic testing for determination of *SMN1/2* copy numbers.
- The combination of above assays reduce the time to diagnosis of SMA.