All-in-one solution for single gene disorders with rare instance

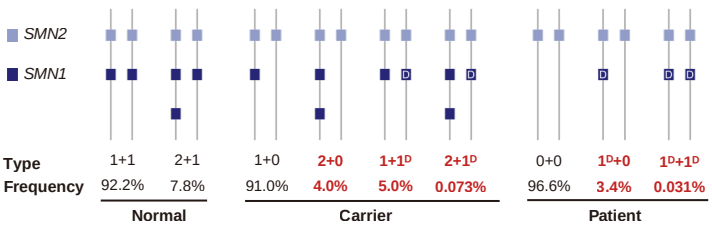
Spinal Muscular Atrophy

Background

Spinal muscular atrophy (SMA) is characterized by muscle weakness and atrophy due to the progressive degeneration and irreversible loss of anterior horn cells in the spinal cord and brainstem nuclei. [1] It is primarily caused by pathogenic mutations in the *SMN1* gene. The highly homologous *SMN2* gene can express approximately 10% of full-length functional SMN protein, which may compensate for the loss of the *SMN1* gene and modify the severity of the disease.

Conventional molecular assays for SMA, such as qPCR and MLPA, are dosage-dependent. These methods target the c.840C variant in exon 7 as a marker for the *SMN1* gene, which does not always accurately represent the actual gene copy number, especially in cases where the *SMN1* gene deletion occurs in other exon regions or in the presence of point mutations.

Conventional genetic assays **fail to detect *SMN1* gene defects caused by small variants** and **cannot identify [2+0] carriers**, who account for **about 9% of carriers and 3% of patients**



Information derived from reference [2-5]
[2+0] carrier: This carrier has 2 functional copies of the *SMN1* gene located on the same chromosome, and absent of the *SMN1* gene on the other chromosome
"D" : Abnormal function of the *SMN1* gene on one chromosome with the presence of variant

Comprehensive Analysis of Spinal Muscular Atrophy (CASMA)

covers the most SMA possibilities in one test

Using single-molecule real-time (SMRT) sequencing, CASMA provides direct interpretation from the full-length sequences of the *SMN1* and *SMN2* genes. This enables accurate determination of functional copy numbers, identification of small variants, and detection of [2+0] carriers. It covers the full spectrum of SMA possibilities, including previously unexplained cases, offering valuable support to clinicians in molecular diagnosis.

Patent (China): ZL 202210234941.6

Number and type of variants		Panel	
SMN1 copy number (full-length)		Basic	Comprehensive
SMN2 copy number (full-length)			
[2+0] carrier (add-on)			
SMN1 variants	133 pathogenic and likely pathogenic variants		
	188 pathogenic, likely pathogenic, and VUS variants		

Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIe system
Sample type: Dried blood spot (DBS), gDNA, blood, and buccal swab
Operation hour: 80 hours
(starting from sample preparation to report generation)

	qPCR	MLPA	NGS	CASMA
***SMN1/SMN2* copy number**	✓	✓	✓	✓
***SMN1* variants**	×	×	×	✓
[2+0] carrier	×	×	×	✓

Clear discrimination of actual functional copy number

The causes of SMA and its subtypes are strongly correlated with the copy number of *SMN1* and *SMN2* genes, as well as causative variants. Conventional genetic assays often rely on exon 7 marker sites, missing causative variants located outside these regions, while next-generation sequencing (NGS) struggles to differentiate mutations between the highly homologous *SMN1* and *SMN2* genes. CASMA overcomes these limitations by providing precise identification of causative mutations and determining the functional copy number, capturing all relevant risks in a single test.

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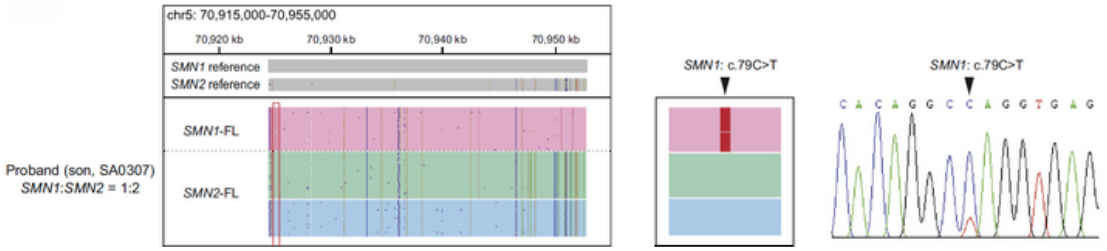
Comprehensive Analysis of Spinal Muscular Atrophy

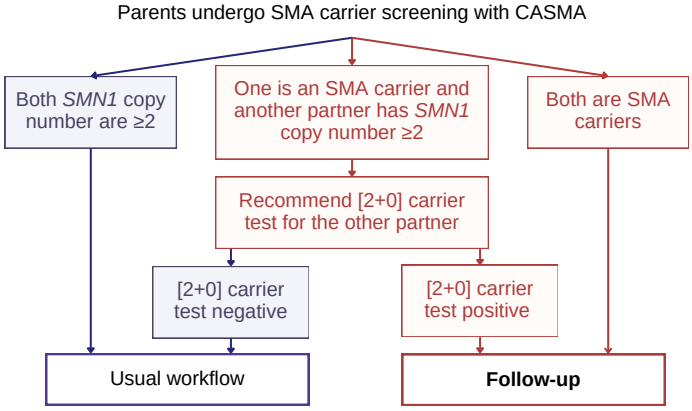
Example 1: CASMA identifies *SMN1:* c.79C>T variant in [1D+ 0] patient



Left and Middle: The *SMN1*: c.79C>T variant in exon 1 identified by CASMA with IGV display; Right: Sanger sequencing confirmed the detected c.79C>T variant. [6]

Identifies [2+0] carrier without proband

CASMA provides an advanced add-on test for detecting [2+0] carriers without the need for a proband. By leveraging haplotype analysis with long-read sequencing through trio analysis, it minimizes the risk of an affected child being born, even in the absence of a known family history.



Example 2: CASMA [2+0] carrier test using trio analysis

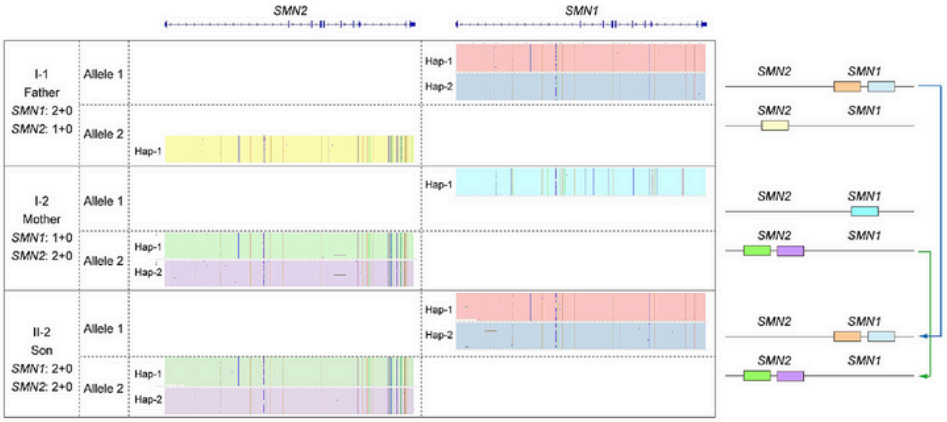


Diagram of CASMA trio analysis with IGV display. Distribution patterns of *SMN1* and *SMN2* on the two alleles detected by CASMA (I-1, I-2, and II-2). Arrows indicated the inheritance of alleles between generations. [7]

Performance backed by published data

In a retrospective study [8] involving 202 clinical samples from 67 families with a family history of SMA or SMA-like conditions, CASMA demonstrated/identified:

- 100% (202/202) accuracy for *SMN1* copy number detection, and corrected *SMN1* copy number from MLPA in 4 samples from 2 pedigrees
- 99.5% (201/202) accuracy* for *SMN2* copy number detection, with clear discrimination between 3 and 4 copies
- 23 SNVs/InDels in *SMN1* and one *SMN2* variant
- the ability to detect [2+0] carriers without the need for a proband

* one sample had no *SMN1* and two *SMN2* copies with the same haplotype, thus the *SMN1:SMN2* copy number were miscalled as 0:1

References: [1] Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: Adam MP, Feldman J, Mirzaa GM, et al., eds. *GeneReviews*. Seattle (WA): University of Washington, Seattle; February 24, 2000. [2] Smith M, Calabro V, Chong B, Gardiner N, Cowie S, du Sart D. Population screening and cascade testing for carriers of SMA. *Eur J Hum Genet*. 2007;15(7):759-766. [3] Qu YJ, Bai JL, Cao YY, et al. Mutation Spectrum of the Survival of Motor Neuron 1 and Functional Analysis of Variants in Chinese Spinal Muscular Atrophy. *J Mol Diagn*. 2016;18(5):741-752. [4] Mailman MD, Hemingway T, Darsey RL, et al. Hybrids monosomal for human chromosome 5 reveal the presence of a spinal muscular atrophy (SMA) carrier with two *SMN1* copies on one chromosome. *Hum Genet*. 2001;108(2):109-115. [5] Medical Genetics Branch of Beijing Medical Association, and Beijing Rare Disease Diagnosis and Protection Association. Expert consensus on the genetic diagnosis of spinal muscular atrophy. *National Medical Journal of China*. 2020, 100(40):3130-31140. [6] Li S, Han X, Zhang L, et al. An Effective and Universal Long-Read Sequencing-Based Approach for *SMN1* 2 + 0 Carrier Screening through Family Trio Analysis. *Clin Chem*. 2023;69(11):1295-1306. [7] Long J, Cui D, Yu C, Meng W. Evaluating the clinical efficacy of a long-read sequencing-based approach for carrier screening of spinal muscular atrophy. *Hum Genomics*. 2024;18(1):110. [8] Bai J, Qu Y, Huang W, et al. A high-fidelity long-read sequencing-based approach enables accurate and effective genetic diagnosis of spinal muscular atrophy. *Clin Chim Acta*. 2024;553:117743.

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