

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

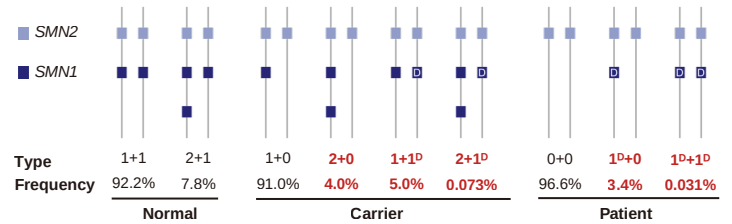
Spinal Muscular Atrophy

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

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 |
|---------------------------------------|------|------|-----|-------|
| <i>SMN1</i> / <i>SMN2</i> copy number | ✓ | ✓ | ✓ | ✓ |
| <i>SMN1</i> variants | × | × | × | ✓ |
| [2+0] carrier | × | × | × | ✓ |

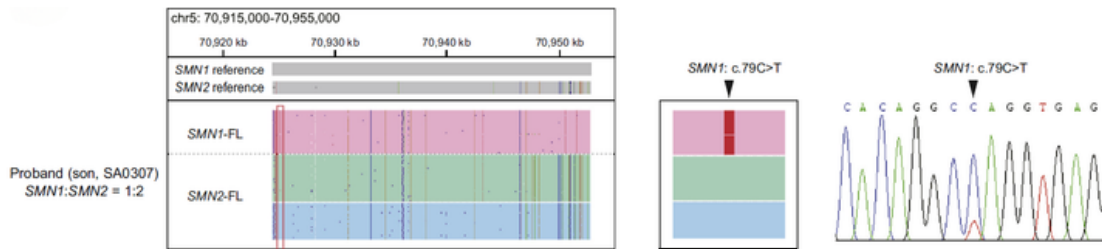
| Number and type of variants | | Panel | |
|---------------------------------------|---|-------|---------------|
| <i>SMN1</i> copy number (full-length) | | Basic | Comprehensive |
| <i>SMN2</i> copy number (full-length) | | | |
| [2+0] carrier (add-on) | | | |
| <i>SMN1</i> variants | 133 pathogenic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

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.

Comprehensive Analysis of Spinal Muscular Atrophy

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

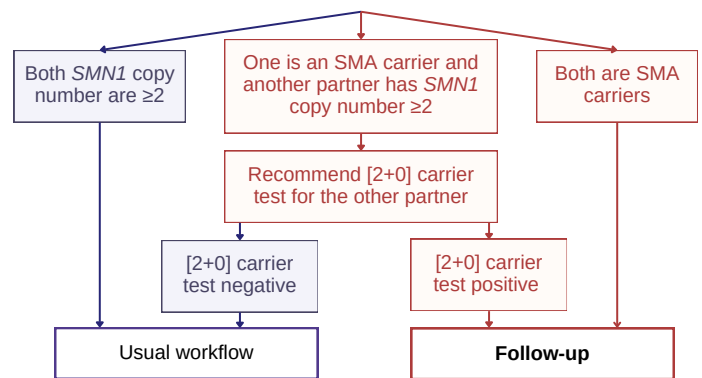


Left and Middle: The *SMN1*: c.79C>T variant in exon 1 identified by CASMA with IGV display; Right: Sanger sequencing confirmed the detected c.79C>T variant. [6]

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.

Parents undergo SMA carrier screening with CASMA



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

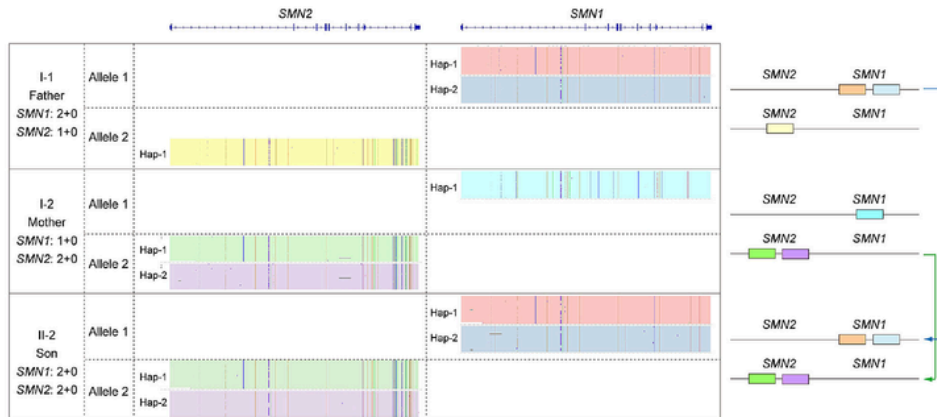


Diagram of CASMA trio analysis with IGV display. Distribution patterns of *SMN1* and *SMN2* on the two alleles detected by CASMA (I-1, I-2, and II-2). Arrows indicated the inheritance of alleles between generations. [7]

Upgraded Performance

In a recent clinical study [8] utilizing 414 retrospective peripheral blood samples and 303 prospective dried blood spots, an updated CASMA demonstrated:

- 100% accuracy in detecting *SMN1* and *SMN2* copy numbers, with clear resolution of *SMN2* high-copy samples (3 vs. 4)
- Simultaneous identification of SNVs and InDels within the genes, and capable of detecting [2+0] silent carriers without proband
- First-pass success rates increased to 99% for long-term stored gDNA and 98.7% for long-term stored DBS, demonstrating the potential for adoption in large-scale newborn screening programs

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 References: [1] Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. In: Adam MP, Feldman J, Mirzaz 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] Li S, Liu B, Zhang J, et al. A More Clinically Effective Long-Read Sequencing-Based Approach for Comprehensive Analysis of Spinal Muscular Atrophy. *J Mol Diagn*. 2026;28(2):136-146.
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