Cleveland Clinic

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Spinal Muscular Atrophy Testing via *SMN1/SMN2* Copy Number Analysis

Background

SMN1-related spinal muscular atrophy (SMA) (OMIM# 253300, 253550, 253400, 271150) is an autosomal recessive neuromuscular disorder, with an incidence of approximately 1 in 10,000 births. The condition has variable severity and age of onset, and has been categorized into clinical types 0-IV. SMA I accounts for 60% of all SMA and has onset of symptoms in infancy. At the most severe end of the spectrum, SMA 0 correlates with prenatal onset of muscular weakness and neonatal respiratory failure, while SMA IV has the mildest presentation and correlates with adult onset of muscle symptoms.

In all types, the genetic cause maps to an inverted duplication on chromosome 5q13.2. (Melki 1990) The complexity of the 5q13 region increases the likelihood of errors during DNA replication, resulting in a relatively high risk of deletions, gene conversions, and new mutations. The survival motor neuron genes, *SMN1* and *SMN2*, are two highly similar genes located in this region. (Lefebvre 1995, Bürglen 1996) One difference between these genes affects protein coding. Alternative splicing of exon 7 in *SMN2* results in decreased production of the full length functional SMN protein compared to *SMN1*. Therefore, *SMN1* accounts for the majority of SMN production, and mutations in *SMN1* are the cause of the SMA phenotype.

Ninety-five percent of patients with SMA have homozygous deletions of the *SMN1* gene [noted as 0+0]. Of the remaining 5% of patients, most are heterozygous for a deletion of *SMN1* on one chromosome and a small pathogenic, or disease-causing, variant in the *SMN1* copy on the other chromosome [0+1] (Wirth 2000).

SMN1 copy number varies among healthy individuals [1+1 or 2+1]. SMA carriers generally have one copy of *SMN1*, while the other copy is deleted [1+0]. However, some people have two, or even three, copies of *SMN1* on the same chromosome. Individuals with two or three copies of *SMN1* may therefore also be carriers if all copies are on the same allele [2+0 or 3+0]. Two-copy *SMN1* alleles are relatively common, with variability among ethnic groups ranging from about 3.6% in Caucasians up to 27.5% in African-Americans. (Sugarman 2012)

Clinical Indications

Carrier Screening: Both the American College of Medical Genetics and Genomics and the American College of Obstetricians and Gynecologists now recommend that SMA carrier screening be offered to all women/couples who are planning a pregnancy or currently pregnant. (Prior 2008, Rink 2017) In individuals with a family history of SMA, it is best to obtain genetic test reports from family members

Ethnicity	Carrier Rate	Detection Rate (%)	Reduced Risk with 2 *SMN1* Copies	Reduced Risk with 3 *SMN1* Copies
Caucasian	1:47	94.8	1:834	1:5600
Asian Indian	1:52	90.2	1:443	1:5400
Asian	1:59	93.3	1:806	1:5600
Ashkenazi Jewish	1:67	90.5	1:611	1:5400
Hispanic	1:68	90.0	1:579	1:5400
African-American	1:72	70.5	1:130	1:4200

Table adapted from *Sugarman et al. 2012*

before testing, to confirm the diagnosis and type of mutation. In a pan-ethnic U.S. population studied by Sugarman et al., the carrier detection rate through *SMN1* copy number, aka dosage, analysis is estimated at 91%. (Sugarman 2012) Ethnicity-specific carrier and detection rates based on this study are provided in the Table at the bottom of page 1.

Diagnostic Confirmation: *SMN1* gene copy number analysis will confirm diagnosis for around 95% of patients with SMA, those with homozygous deletions of *SMN1* [0+0]. In patients with one copy of *SMN1* in whom there is a high suspicion of SMA, *SMN1* sequencing should be considered to look for a small variant or deletion.

Because the *SMN2* gene produces a small amount of SMN protein, increased copy number of this gene is inversely correlated with SMA disease severity. (Mailman 2002) Variation in the number of *SMN2* copies is in large part responsible for the variation seen among different types of SMA. However, since other factors also influence severity, definitive genotype-phenotype predictions cannot be made based solely on *SMN2* copy number. (Prior 2011)

Methodology

DNA is purified from peripheral blood and the *SMN1* (NM_000344.3) and *SMN2* (NM_017411.3) genes are interrogated by multiplex fluorescent polymerase chain reaction followed by capillary electrophoresis. The test detects *SMN1* copy number (by detecting dosage at exon 7), with analytic sensitivity of >99% and clinical sensitivity of 95% for SMA patients. Sensitivity for carrier screening depends on patient ethnicity, as seen in the Table. Analytical and clinical specificity is >99% for use in both diagnostic testing and carrier screening. The test also detects *SMN2* copy number (also by detecting exon 7 dosage), which influences severity of disease in affected patients but does not impact carrier status. (Prior 2011)

Limitations

This laboratory developed test does not detect single sequence variants or small deletions/duplications within *SMN1*. In individuals found to have two copies of *SMN1*, the test cannot determine whether those copies are on the same [2+0] or opposite [1+1] chromosomes. This impacts the sensitivity of carrier screening, as seen in the Table. In the case of a two-copy allele, the individual is a "silent carrier" and has a risk of passing on an allele that is deleted for *SMN1*. A further limitation of reproductive risk assessment is the high rate of *de novo SMN1* mutations; 2% of SMA patients have new mutations that were not inherited. (Wirth 1997)

Interpretation

Thorough interpretation of results is dependent on the indication for testing and relies on good communication of clinical information from the ordering provider. In healthy individuals without a family history of SMA, detection of two *SMN1* copies reduces (but does not eliminate) the risk of being a carrier, since sensitivity of detection using copy number analysis is lower among some ethnicities. Detection of 3 *SMN1* copies further reduces the carrier risk. *SMN2* copy number is not relevant for carrier status.

Among patients with clinical presentations suggestive of SMA, detection of zero *SMN1* copies confirms the diagnosis. In symptomatic patients with one *SMN1* copy, *SMN1* gene sequencing should be considered to identify the small percentage of patients with heterozygous sequence variants or small deletions. Symptomatic patients with two *SMN1* copies are unlikely to have SMA, though very rare cases of homozygous sequence variants have been reported.

References

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Test Overview

Test Name	Spinal Muscular Atrophy Carrier Screening and Diagnostic Assay
Mnemonic	SMAGEN
Methodology	Multiplex fluorescent polymerase chain reaction and capillary electrophoresis
Specimen Requirements	4 mL peripheral blood, EDTA (lavender) transported and stored ambient up to 24 hours. Due to difficulties associated with newborn and infant draws, smaller volumes may be submitted (minimum of 0.5mL is required).
CPT Codes	81329

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