TITLE: DETERMINING THE CLINICAL UTILITY OF DNA AND RNA TESTING OF *SMN* IN PATIENTS CLINICALLY AFFECTED WITH SPINAL MUSCULAR ATROPHY.

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ABSTRACT

Spinal muscular atrophy (SMA) is a common neuromuscular disorder occurring as frequently as albinism in the black South African (SA) population. SMA is a neurodegenerative disease characterised by motor neuron loss in the spinal cord causing muscle weakness and atrophy. SMA is caused predominantly by mutations in the survival motor neuron 1 gene (*SMN1*). A homozygous deletion of *SMN1*, exon 7 is the main cause of SMA in ~95% of patients worldwide but only occurs in 51% of black SA patients. Mutations within *SMN2,* a gene copy, are not thought to cause SMA directly, but to modify disease severity. Black SA individuals have been shown to have copy number variations of the *SMN1* and *SMN2* genes which could potentially mask pathogenic mutations. The aim of the study was to determine the clinical utility of different DNA and RNA methods in testing clinically suggestive SMA patients without an *SMN1* deletion. Three new methods, AmplideX® PCR/CE SMN1/2, qPCR and RT-qPCR were optimised and validated as part of this study. Ninety-two subjects were tested using these methods and results were compared to those obtained with Multiplex Ligation-Probe Dependent Amplification (MLPA). Comparative analysis of the three methods indicated that they were all adequate for diagnostic and carrier testing of SMA subjects. The RT-qPCR showed that DNA copy number does not necessarily correlate directly with RNA copy number. Interpreting expression of the *SMN*Δ7 transcript must be done in the context of the *SMN1* and *SMN2* DNA and RNA copy numbers. DNA copy number detection by qPCR was the most affordable method and AmplideX® PCR/CE SMN1/2 was the only method able to detect gene conversions, although the functional significance of these is uncertain. Diagnosis in subjects with clinical features suggestive of SMA could not be confirmed with RT-qPCR. We recommend further clinical assessment of these patients and testing using newer technologies. Further research into the molecular mechanism underlying SMA in patients with African ancestry is required.