Detection of SMN1 deletions by a simple fluorescent multiplex PCR method

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INTRODUCTION

With a prevalence of about 1/6000 and a carrier frequency of 1/401/60, the proximal spinal muscular atrophies (SMAs) are among the most frequent autosomal recessive hereditary disorders. The SMA 5q13 region shows a complex structure at the genomic level, including a 500-bp duplication and inversion. The survival motor neuron (SMN) gene is present in at least one telomeric (SMN1) and one centromeric copy (SMN2) per chromosome in normal (non-carrier) individuals. The duplication of the SMA locus makes the detection of SMA carriers difficult, and this has hampered genetic counseling in affected families.

AIM OF THE STUDY

Detection of heterozygous SMN1 deletions in Macedonian SMA Familiesin five thrombophilic genes (C677T and A1298C in MTHFR gene, FII G20210A, FVLeiden, FV A1702C and A1299G, PAI-I 4G/5G and FXIII G34T) and subsequent analysis on ABI 310 genetic analyzer (Figure 2).

MATERIALS

DNA samples from 56 parents of patients with SMA and from 35 control individuals were extracted from leucocytes by phenol/chlorophorm extraction procedure.

METHODS

SMN1 Copy-number assay

Two competitive amplifications are performed in a multiplex PCR reaction. We simultaneously amplified exon 7 of the SMN1 and SMN2 genes using a mismatch primer X7-Dra, which introduced a DraI restriction site into amplified SMN1 exon 7 and RB exon 13, which contains a DraI restriction site. The PCR reaction was performed in a final volume of 25 µl, using 0.5 µmol/l SMN primers, 0.25 µmol/l RB exon13 primers, 0.2 mmol/l dNTP, 1.5 mmol/l MgCl2, 1 unit of Taq polymerase (AmpliTaq Gold Applied Biosystems), and 100 ng of genomic DNA. The PCR consisted of 25 cycles of 940 C for 45 seconds, 570C for 60 seconds, and 720C for 90 seconds, proceeded by an initial denaturation step of 10 minutes at 940C and followed by a final extension of 10 minutes at 720C. The entire PCR reaction was then digested using four units of DraI (New England Biolabs) in a total volume of 15 µl for at least four hours. Digested samples were analyzed by capillary electrophoresis on ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The size of the peak was determined by measuring its peak area. Given that there are two copies of the RB gene, the relationship between SMN1/RB is used to determine the relative number of copies of SMN1-gene. Completeness of DraI digestion was monitored by absence of undigested products of 237 bp (RB exon 13 product). The genomic SMN1/genomic RB ratio was used to determine the relative copy number of SMN1 for all samples.

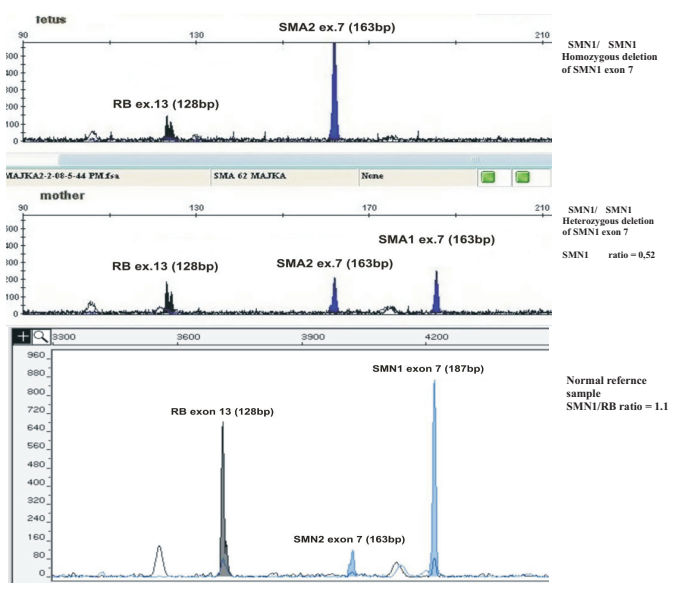

Figure 1. SMN1 dosage analysis of SMA patient a spinal muscular atrophy carrier and a normal control. Line 1: homozygous deletion of SMN1 exon 7. Line 2: heterozygous (one copy of SMN1) for the deletion of SMN1 exon 7. Line 3: .normal sample with two copies of

RESULTS

In 54 out of 56 samples from parents of multiple-affected children with SMA, a SMN1/RB ratio compatible with a single SMN1 exon 7 copies was found. A representative analysis is shown in Figure 1. The average ratio observed in these 54 samples was 0.54 ranging from 0.27 to 0.70. In two of 56 samples a SMN1/RB ratio compatible with two SMN1 exon 7 copies was found.

Non-carriers have SMN1/RB ratio over 0.75. In 35 samples from normal individuals, a SMN1/RB genomic ratio compatible with two SMN1 exon 7 copies was found. The average of this ratio observed in these 35 samples was 1.2 ranging from 0.78 to 1.79.

CONCLUSION

Our results failed to support the relationship between MTHFR C677T and A1298C polymorphisms and the risk of having a child with aneuploidy.

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