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Confirmation diagnostics of positive cases in spinal muscle atrophy (SMA) newborn screening using MLPA analysis (reliability of SMN2 copy number determination , timeline)

Introduction: Spinal muscular atrophy (SMA) is the most common neurodegenerative disease in childhood with an incidence of about 1:7,000 in Germany. About 95% of patients show a homozygous deletion of exon 7 in the SMN1 gene. Newborn screening for SMA is based on the detection of the homozygous absence of exon 7 in the SMN1 gene by allelic-specific PCR analysis from the dried blood spots. Confirmation diagnostics and the determination of the SMN2 copy number in positive cases was performed with DNA isolated from a second blood sample.

Methods: Genomic DNA was extracted from EDTA blood samples using the FlexiGene DNA Kit (Qiagen). MLPA analysis (multiplex ligation-depended probe amplification) was carried out according to the manufacturer`s protocol (MRC-Holland, The Netherlands) using the SALSA Probe Mix P021-A2. MLPA is a semiquantative testing method used for determination of the SMN1 and SMN2 copy number in the patients' DNA samples. This probemix contains one specific probe each for SMN1 exon 7 and exon 8, and also for SMN2 exons 7 and 8.

Results: In all 71 positive cases the homozygous absence of the exon 7 of the SMN1 gene was confirmed via by MLPA analysis. In addition to the confirmation, the SMN2 exon 7 copy number was determined by semiquantitative analysis. The following SMN2 copy numbers were identified: 2 copies in 31 samples, 3 copies in 18 samples, 4 copies in 20 samples and 5 copies in 2 samples.

Conclusion: The sensitivity and specificity in SMA newborn screening seems to be at a very high level. MLPA is an appropriate method for the confirmation of the homozygous deletion detected in newborn screening as well as for the simultaneous determination of the SMN2 copy numbers that is essential for the selection of the available therapy options. Therefore, this confirmation diagnostics should be carried out promptly, preferably within one week. The accurancy of SMN2 copy number determination is very high and reliable using the approved MLPA kit.

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