CONGRESUL CONSACRAT ANIVERSĂRII A 75-A DE LA FONDAREA USMF „NICOLAE TESTEMIȚANU”
20 23 octombrie 2020

DIAGNOSIS OF SPINAL MUSCULAR ATROPHY THROUGH QPCR METHOD

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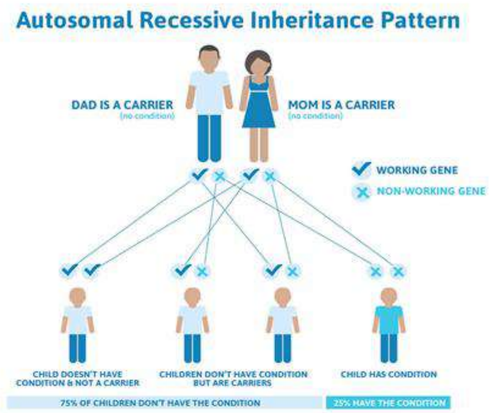
Introduction

Spinal muscular atrophy (SMA) is a progressive neuromuscular disease inherited in an autosomal recessive way. The prevalence of SMA in the RM constitutes 8.43 ±0,15:100000 population. 95% of SMA is caused by deletion of exon 7 of SMN1. In carrier couples there is a 25% chance of offspring with SMA.



Keywords: molecular genetics, diagnosis, method, screening, SMA

Purpose

Diagnosis of SMA trough qPCR method (caused by deletion of exon 7 SMN1) in Human Molecular Genetics Lab. This will reduce the time of diagnosis and offer the possibility to identify the carriers of deletion.

Material and methods.

60 DNAs representing 15 couples from control group and 10 families (mother, father and child affected or suspected) were diagnosed for determining the status of exon 7 SMN1 by qPCR method, melting curve (2 replicates for SMN1 exon 7, 1 replicate for the ALB exon 12). The DNA concentration was measured by spectrophotometry. EvaGreen was used as a DNA-binding dye.

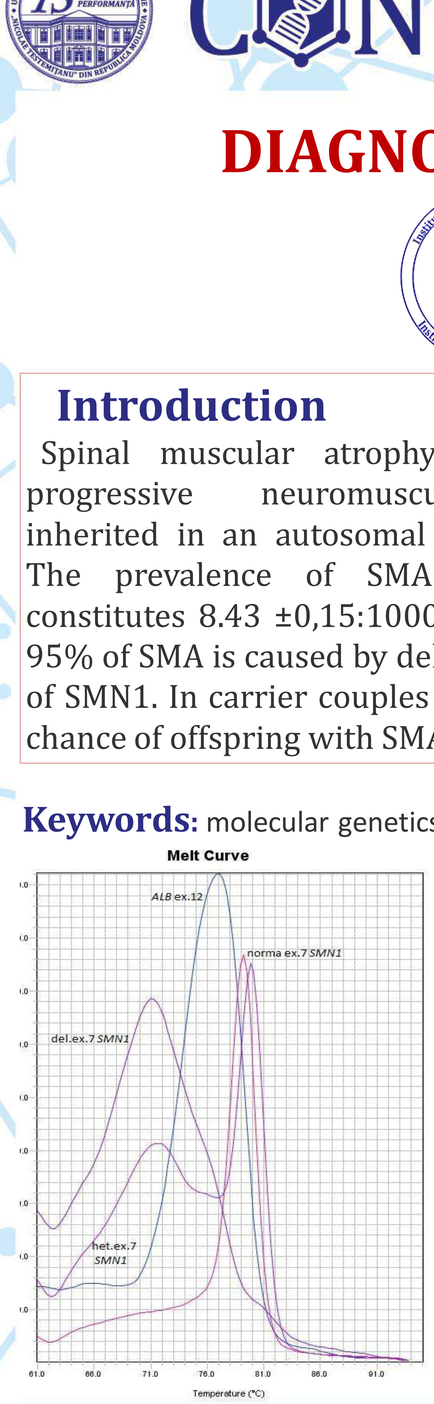


Fig. 1: Melt curve profile for each status of SMN1 7 exon

Results

Diagnosis of SMA is available through different methods. The molecular genetic diagnosis by PCR-RFLP is expensive and time consuming than qPCR method. For all DNA samples, amplification occurred for both exon 12 ALB and exon 7 SMN1. According to the melting curves, in families with history of SMA 9 DNAs with heterozygous status were identified and 7 DNA with exon deletion 12 have the status normal for exon 7 gene SMN1 and for 2 DNAs the reaction did not take place. For 22 persons from control group the exon7 SMN1 was determined to be present and for 8 persons was determined heterozygous status (5 women and 3 men). Among those who are heterozygous, 2 people form the same couple.

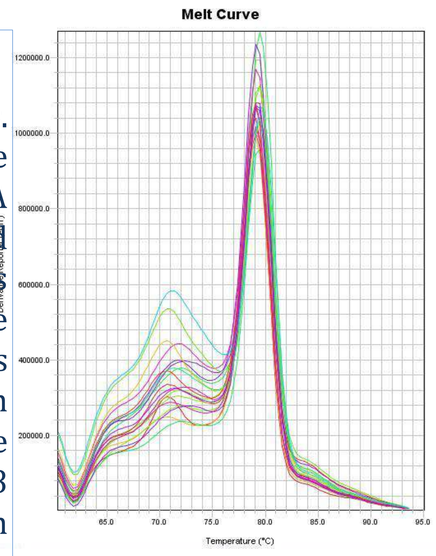
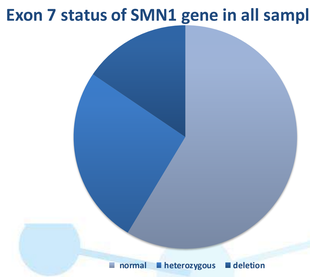


Fig. 2: Melt curve profile for normal exon 7 *SMN1* status



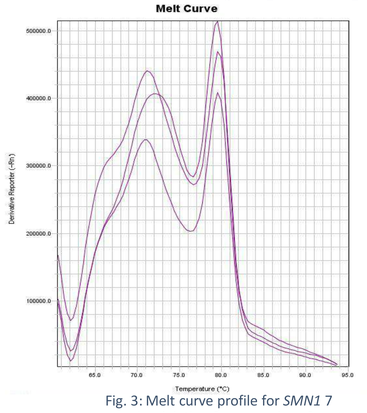


Fig. 3: Melt curve profile for *SMN1* 7 exon carrier deletion from couples of control group

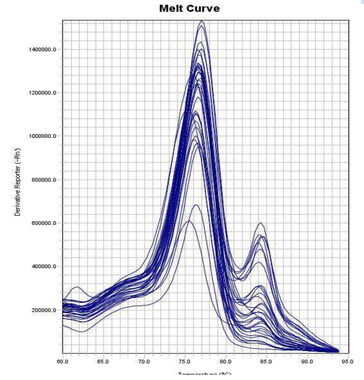


Fig. 4: Melt curve profile for *ALB, 12* exon of all samples

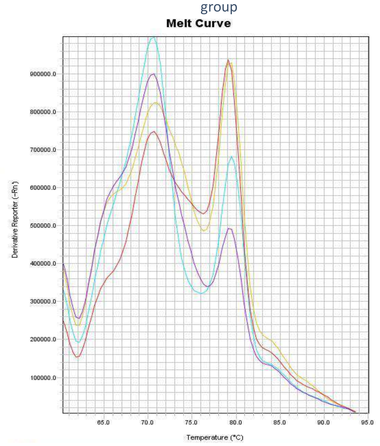


Fig.5: Melt curve profile for *SMN1* 7 exon carrier deletion from families with historic of SMA

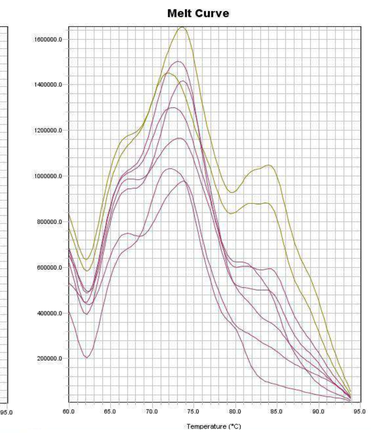


Fig. 6: Melt curve profile for *SMN1* 7 exon deletion of probands from families with historic of SMA

Conclusions

Considering the presence of treatment the diagnosis as soon as possible is needed and QPCR is an effective method for this: prenatal for families in which the history of SMA is already present, for newborns (newborn screening) and even in the family planning process (carrier screening).

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