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DETERMINATION OF DELETION CARRIER OF EXON 7 OF THE SMN1 GENE BY THE QPCR METHOD

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REZUMAT

DETERMINAREA PURTĂTORULUI DE DELEȚIE AL EXONULUI 7 AL GENEI SMN1 PRIN METODA QPCR

Cuvinte cheie: genetică moleculară, diagnostic, deleție, purtător, screening, SMA.

Introducere. Atrofia musculară spinală (SMA) este o boală neuromusculară progresivă moștenită într-un mod autozomal recesiv. Prevalența SMA în Republica Moldova constituie 8,43 ± 0,15: 100000 populație, care este semnificativ mai mare decât datele din Orphanet (2,93) și Rusia (0,31-3,91). Frecvența purtătorilor a fost estimată la 1: 40-1: 60. SMA este cauzată în 95% din cazuri de deleția exonului 7 al genei SMN1. Dacă ambele persoane dintr-un cuplu sunt purtătoare de această deleție, acest cuplu are riscul de 25 % de a avea un copil afectat. Scopul nostru a fost de a dezvolta un sistem pentru a detecta deleția homozigotă și heterozigotă a exonului 7*, SMN1* prin metoda PCR în timp real.

Materiale și metode. A fost efectuat diagnosticul molecular-genetic pentru identificarea delețiilor în gena SMN1 la 25 de familii moldovenești: 10 copii afectați și părinții acestora, 20 de persoane din familii cu SMA și alți 30 de pacienți din grupul de planificare familială, fără istoric al SMA. Am stabilit un sistem simplu folosind probe de ADN și tehnica PCR în timp real. Concentrația ADN a fost măsurată prin spectrofotometrie.

Rezultate. Conform curbelor de topire, în familiile cu antecedente de SMA, au fost identificate 9 ADN-uri cu statut heterozigot al exonului 7, SMN1; 7 ADN cu deleție homozigotă a exonului și 12 probe au forma normală. Pentru 22 de persoane din grupul de control al planificării familiale a fost identificată forma normală homozigotă a exonului 7 SMN1, iar pentru 8 persoane s-a determinat statutul heterozigot (5 femei și 3 bărbați). Dintre cei care sunt heterozigoți, 2 persoane formează același cuplu.

Concluzii. Testul elaborat este eficient pentru diagnosticul delețiilor exonului 7, SMN1, fapt confirmat de asocierea cu rezultatele obținute prin standardul de aur PCR-RFLP. Plus el adăugă posibilitatea de a identifica și purtătorii heterozigoți ai delețiilor exonului 7 ai SMN1. În ciuda limitărilor, testul poate fi folosit ca instrument de identificare a celor mai frecvent întâlnite deleții cauzatoare de SMA.

РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ НОСИТЕЛЯ ДЕЛЕЦИИ 7 ЭКЗОНА ГЕНА SMN1 МЕТОДОМ QPCR

Ключевые слова: молекулярная генетика, диагностика, ПЦР носительство, скрининг, СМА.

Введение. Спинальная мышечная атрофия (СМА) - прогрессирующее нервно-мышечное заболевание, наследуемое по аутосомно-рецессивному типу. Распространенность СМА в Республике Молдова составляет 8,43 ± 0,15 на 100 000 населения, что значительно выше данных Orphanet (2,93) и России (0,31–3,91). Частота носительства оценивалась как 1: 40–1: 60. В 95% случаев СМА вызывается делецией 7 экзона гена SMN1. Если оба человека в паре гетерозиготны, риск рождения больного ребенка у этой пары составляет 25%. Нашей целью было разработать систему для обнаружения гомозиготной и гетерозиготной носительство делеции экзона 7, SMN1, с помощью ПЦР в реальном времени.

Материалы и методы. Молекулярно-генетическая диагностика для выявления делеций в гене SMN1 была проведена в 25 молдавских семьях: 10 больных СМА и 20 родителей больных детей и еще 30 пациентов из группы планирования семьи без СМА в анамнезе. Мы создали простую систему, используя технику ПЦР в реальном времени и образцы ДНК. Концентрацию ДНК измеряли спектрофотометрически.

Полученные результаты. Согласно кривым плавления, в семьях с историей SMA было идентифицировано 9 ДНК с гетерозиготным статусом экзона 7, SMN1; 7 ДНК с гомозиготной делецией экзона и 12 образцов имеют нормальную форму. У 22 человек в контрольной группе планирования семьи была идентифицирована нормальная гомозиготная форма экзона 7 SMN1, а у 8 человек определен гетерозиготный статус (5 женщин и 3 мужчины). Из тех, кто является гетерозиготным носителям, 2 человека образуют одну пару.

Выводы. Разработанный тест эффективен для диагностики делеций экзона 7, SMN1, что подтверждается ассоциацией с результатами, полученными с помощью золотого стандарта ПЦР-ПДРФ. Кроме того, он добавляет возможность быстрого выявления гетерозиготных носителей делеций экзона 7 SMN1. Несмотря на ограничения, тест можно использовать как диагностический инструмент для выявление частых делеций, вызывающих СМА.

Keywords: molecular genetics, diagnosis, deletion, qPCR, carrier, screening, SMA.

Introduction. Spinal muscular atrophy (SMA) is a progressive neuromuscular disease inherited in an autosomal recessive way. Spinal muscular atrophy disorder is characterized by degeneration of motor neurons of the spinal cord leading to progressive muscular weakness [1]. SMA cause most common genetic infant mortality with the incidence of 1 in 6,000-10,000 live births [2]. The carrier frequency for the disease was estimated at 1:40-1:60 with some variations among different ethnic groups[3]. The prevalence of SMA in the Republic of Moldova constitutes 8.43 ±0,15:100000 population which is significantly higher than the data from Orphanet (2,93) and Russia (0,31- 3,91)[4]. SMA is linked to the 5q13 locus in 95% of patients, and in at least 98% of them, the *SMN1* homozygous deletion is found [11]. If both persons in a couple are carriers, they have a 1-in-4 chance of having a child with disease [1].

SMA is classified into five clinical types (0-IV). Type 0 SMA presents with severe weakness, hypotonia, and respiratory distress at birth [1]. Type I SMA (Werdnig-Hoffmann disease) presents before 6 months of age with profound hypotonia, symmetrical flaccid paralysis, and no control of head movement [5]. Type II SMA presents between 7 and 18 months of age, and although most individuals can initially sit unaided, none can walk. Type III SMA (Kugel-Welander disease) presents after 18 months of age with considerable disease heterogeneity[6]. Type IV SMA presents as minor muscle weakness in the second or third decade of life[7],[8].

SMA is caused by mutations in the *SMN1* gene (OMIM 600354) located in the telomeric region of chromosome 5q13[9],[10]. Because SMA is one of the most common lethal genetic disorders, with a carrier frequency of 1/40 to 1/60[15], direct carrier testing has been beneficial to many families with affected children. Currently, only individuals with a family history of SMA are routinely being offered carrier testing. The goal of population based SMA carrier screening is to identify couples at risk for having a child with SMA. Since 2016 new therapies have advanced in clinical trials for treatment of SMA and the Food and Drug Administration (FDA) has approved the only treatment for SMA which is called Spinraza (Nusinersen). Later in 2019, a second treatment called Zolgensma (based on *SMN1*-gene therapy) has been approved by regulatory agencies in multiple countries. For an early diagnosis and the initiation of treatment, newborn screening or carrier screening may be useful, and they are feasible because most of the patients lack *SMN1* [13],[17],[18].

Aim. Our aim was to develop a system based on real time polymerase chain reaction (qPCR) technique to detect homozygous and heterozygous *SMN1* exon 7 deletion.

Materials and methods. The adult patients and parents of all children gave written informed consent to the diagnostic procedures. Molecular testing for deletion in the *SMN1* gene was performed in 25 Moldavian families including: 10 affected individuals and their parents, 20 persons, from families with SMA, and 30 other patients from the family planning group, without historic of SMA. We have established a simple system using ADN samples and real-time PCR technique. The DNA concentration was measured by spectrophotometer. Primers were diagnosed to identify exon 7 of the *SMN1* gene and primers to identify exon 12 of *ALB*. *Alb*umin (*ALB*, a housekeeping gene) was used as an internal control of the presence of the amplification process. Previously, samples from families with SMA history that are included in this study were diagnosed for homozygous deletion of exon 7 *SMN1* by PCR-RFLP method.

Results and discussions. For most of DNA samples, amplification occurred for both: exon 12, *ALB* and exon 7, *SMN1*, only for 2 probes of DNAs the reaction did not take place. According to the melting curves (Fig. 1), in families with history of SMA, were identified 9 DNAs with heterozygous status of 7 exon, *SMN1*; 7 DNA with homozygous exon deletion and 12 samples have the wild form of 7 exon of *SMN1*. For 22 people from family planning control group was identified the exon 7 *SMN1* homozygous wild form, and for 8 persons was determined heterozygous status (5 women and 3 men). Among those who are heterozygous, 2 people form the same couple.

SMA affects individuals of all ethnicities. The molecular diagnosis of the SMA consists of the detection of the absence of exon 7 of the *SMN1* gene. The homozygous absence of detectable *SMN1* in SMA patients is being

used as a powerful diagnostic test for SMA[14]. Although the targeted mutation analysis trough PCR RFLP has an excellent sensitivity of approximately 95% in identifying affected homozygote's, it cannot detect SMA carriers who have heterozygous deletions of *SMN1*. Preconception carrier screening allows carrier couples to consider the fullest range of reproductive options[16].

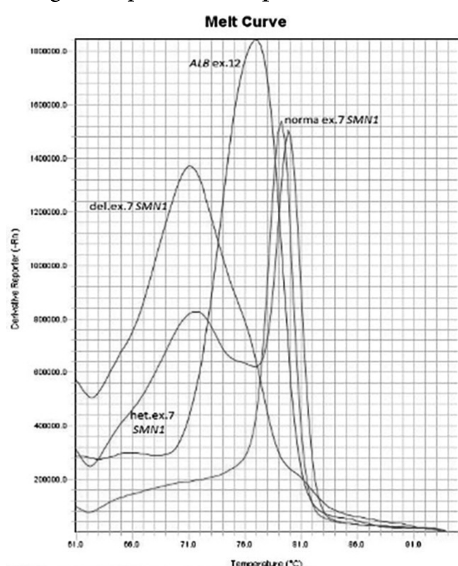

Fig. 1. ***Melt curve profile for each status of SMN1 7 exon, and ALB 12 exon profile***

The choice to have a SMA carrier test should be made by an informed decision[19],[20]. It is important for couples to understand the type and need of testing[21]. Because SMA is the result of a common single deletion event in 95% of the cases, the carrier test is sensitive (~90% detection rate). However, the molecular testing does not identify all carriers and therefore false-negative can occur[12]. Approximately 5% of affected patients are compound heterozygotes, exhibiting a deletion and a point mutation[14],[22]. It is well known that a false-negative result in SMA carriers occurs when the carrier has two *SMN1* genes in cis on the one chromosome 5. Further, approximately 2% of affected individuals have a de novo mutation[23],[24]. The PCR-RFLP test does not detect heterozygous *SMN1* loss and cannot be used for identifying healthy carriers. The test developed by us offers the possibility to determine the deletion carriers of exon 7, *SMN1*. In our study, people from general population group, that form seven couples do not carry a deletion of exon 7, *SMN1*, this assumes that they have 2 copies of the *SMN1* gene. The finding of normal two *SMN1* copy dosage significantly reduces the risk of being a carrier; however, there is still a residual risk of being a carrier and subsequently a small recurrence risk of future affected offspring for individuals with 2 *SMN1* gene copies. One couple with no family history of SMA from this control group was identified as carrier with a 25% risk to have an affected child. Also, other 6 samples were identified to carry a deletion. This people have 95 % of possibility to have a child without SMA in couples that they form now. Among individuals with a family history of SMA, trough PCR-RFLP method was identified that all children have homozygous deletion of exon 7, *SMN1*. Our system, using qPCR, melting curve program, to detect heterozygous *SMN1* exon 7 deletion was established that for approximatively 50 % of this families, the cause of disease was carrier parents. It should be noted that the test developed cannot determine the status of carriers of genotype "2 + 0". Thus, of the remaining percentage, some families with affected children could carry this genotype. Another cause of the presence of the sick child in the family, and the parents are determined not to be carrier of exon 7 deletion, may be the de novo mutation in the children, or they inherited in a compound way some point mutations from the parents.

Conclusion. The developed test is effective for the diagnosis of deletions of exon 7, *SMN1*, fact confirmed by the association with the results obtained by the gold standard PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism). In addition, he added the possibility to identify the heterozygous carriers of deletions of exon 7 of *SMN1*. Despite the limitations, the test can be used as a tool to identify the most common deletions that cause SMA.
Considering the presence of treatment, the diagnosis as soon as possible is needed and QPCR is an effective method for:

- prenatal for families in which the history of SMA is already present,
- for the family planning process (carrier screening),
- for newborns (newborn screening).

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