Korean J Lab Med 2010;30:93-6
DOI 10.3343/kjlm.2010.30.1.93

Determination of *SMN1* and *SMN2* Copy Numbers in a Korean Population using Multiplex Ligation-dependent Probe Amplification

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Determination of the copy number of the survival motor neuron (*SMN*) gene is important for detecting spinal muscular atrophy (SMA) carriers and compound heterozygous patients. Multiplex ligation-dependent probe amplification (MLPA) assay is a simple and efficient technique used for detecting variations in the copy numbers of different genes. Race- and ethnicity-based variation in the SMA carrier frequency and the '2+0' genotype of *SMN1* are important factors that should be considered when estimating the risk of being an SMA carrier. Since *SMN2* plays a disease-modifying role, accurate determination of *SMN2* copy numbers in SMA patients can serve as a useful prognostic tool. Therefore, information on the *SMN2* genotype distributions in normal populations will be helpful in selecting appropriate reference samples for MLPA analysis. To determine SMA carrier frequencies and *SMN* genotype distribution, we determined the copy numbers of *SMN1* and *SMN2* genes using the MLPA assay in 100 unrelated Korean individuals with no family history of SMA. The frequency of SMA carriers in the Korean population appears to be 1 in 50, which indicates that the prevalence of SMA among Koreans is the same as that among individuals in the Western countries. Two of the 100 normal individuals enrolled in this study showed 3 copies of the *SMN1* gene. Therefore, 1.0% of the 198 normal alleles in this population was estimated to be 2-copy alleles ('2+0' genotype). *SMN2* copy numbers showed a high degree of individual variation. Our results showed that 64% of the individuals had 2 copies of *SMN2*, but 36% individuals had between 0, 1, or 3 copies of the gene. *(Korean J Lab Med 2010;30:93-6)*

Key Words : *Spinal muscular atrophy, Multiplex ligation-dependent probe amplification, Carrier, Copy number, Survival motor neuron gene, Koreans*

Spinal muscular atrophy (SMA), a disease characterized by the degeneration of the anterior horn cells of the spinal cord, causes symmetric proximal muscle weakness. In approximately 95% of cases, SMA is caused by the homozygous deletion of the survival motor neuron 1 (*SMN1*) gene (5q13) or its conversion to *SMN2* [1]. The prevalence of SMA, an autosomal recessive disease, is approximately 1 in 10,000 newborns [2]. Hendrickson et al. [3] reported that the carrier frequency of *SMN* mutation was different among different ethnic groups in North America: the carrier frequency was 2.7% in Caucasians, 1.8% in Asians, 1.1% in African Americans, and 0.8% in Hispanics. The carrier frequency of the later 2 groups was lower than that of the former groups. Copy number analysis of the *SMN1* gene is important to identify carriers with *SMN* mutation. The American College of Medical Genetics (ACMG) recommends universal screening for the presence of SMA mutation to identify carriers because this condition is as-

Received : September 4, 2009 Manuscript No : KJLM09-112
Revision received : November 20, 2009
Accepted : November 20, 2009
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*This study was supported by Konkuk University.

sociated with clinically severe presentation and a high carrier frequency [4]. Since SMA occurs in all populations, regardless of race or ethnicity, carrier testing should be recommended to all couples considering pregnancy or in early stages of pregnancy. However, carrier testing for SMA, using *SMN1* gene analysis, has not been considered as a part of routine clinical investigation in such couples in Korea.

SMN1 gene produces full-length transcripts only whereas the majority of *SMN2* transcripts lack exon 7 due to alternative splicing. In normal subjects, most of the functional SMN protein is a result of the *SMN1* gene, with only a very small amount coming from the *SMN2* gene. In more than 95% of patients, SMA is due to the deletion of *SMN1* or its conversion into *SMN2*, while in about 5% of the patients, the disease is caused by intragenic point mutations of *SMN1*. *SMN2* has been reported to play a disease-modifying role because the *SMN2* copy numbers inversely correlate with the severity of the SMA phenotype. Various methods have been reported for determining the copy numbers of the *SMN* gene; these methods include denatured high-performance liquid chromatography (DHPLC) and real-time quantitative polymerase chain reaction (PCR) [5-8]. Real-time PCR is difficult to set up because the multiplexing required to evaluate a useful number of loci can be difficult to optimize. DHPLC has been successfully used in screening for SMA carriers; however, when performed by inexperienced technicians, this technique yields inconsistent results. The multiplex ligation-dependent probe amplification (MLPA) assay is efficient in the detection of copy number changes in various genes [3]. Reports indicate that the MLPA assay is better and more efficient than the 2-step DHPLC. The MLPA assay provides the unique ability to hybridize several probes specific for *SMN1* and *SMN2* genes in a single experiment [9].

Although SMA is considered to be a panethnic disease, the epidemiologic data for a specific ethnic group should be collected since the frequency of the carriers can significantly differ among various ethnic groups [3, 10]. We assessed the copy numbers of *SMN1* and *SMN2* genes among Koreans using the MLPA assay to estimate the SMA carrier frequencies and to determine *SMN* genotype distributions that are required for genetic risk assessment.

We collected blood samples from 100 unrelated Korean individuals who had no family history of SMA after written informed consent was obtained from each subject. MLPA analysis was performed using kits P021 according to the recommendations of the manufacturer (MRC Holland, Amsterdam, Netherlands). The PCR products were analyzed by using a 310 ABI sequencer (Applied Biosystems, Foster City, CA, USA). Analysis was performed using the GeneMarker software, version 1.6 (Softgenetics, State College, PA, USA).

The results were classified according to the copy numbers of *SMN1* and *SMN2* (Table 1). The frequency of SMA carriers (2.0%) determined in this study is similar to that observed in previous studies conducted in Western countries and Korea [3, 5, 10]. The copy number of *SMN1* can vary on a chromosome. Two of the 100 individuals investigated in this study showed 3 copies of the *SMN1* '2+1' genotype (Fig. 1). Therefore, 198 were normal alleles (including 2 normal alleles in the individuals with 1 copy of *SMN1*, 192 normal alleles in the individuals with 2 copies of *SMN1*, and 4 normal alleles in individuals with 3 copies of *SMN1* (the assuming 2+1 genotype)); this shows that 1% of the alleles of these 198 normal alleles were of the '2+0' genotype.

Thus, a carrier may possess 2 copies of the gene on 1 chromosome and have 0 copies on another chromosome,

Table 1. *SMN1* and *SMN2* copy numbers in a Korean population

SMN1:*SMN2* ratio	No. of subjects	Interpretation
1:1	1	SMA carrier
1:3	1	SMA carrier
2:0	2	Noncarrier
2:1	28	Noncarrier
2:2	63	Noncarrier
2:3	3	Noncarrier
3:2	1	Noncarrier
2:2/3:1*	1	Noncarrier
Total	100	

*Hybrid *SMN* genes, *SMN1* exon 7:*SMN2* exon 7/*SMN1* exon 8:*SMN2* exon 8.
Abbreviations: SMN, survival motor neuron; SMA, spinal muscular atrophy.

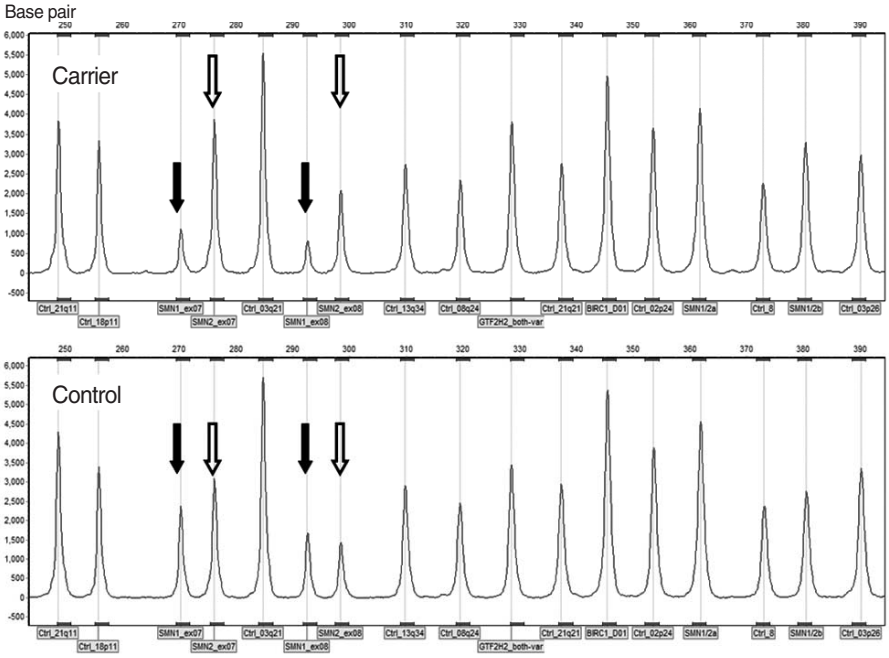

Fig. 1. Electrophoreogram of *SMN1*:*SMN2*=1:3. The carrier is heterozygous for the deletion of the *SMN1* gene as shown by reduction in peak heights from 2 probes for exons 7 and 8 (closed arrows). The relative peak height ratios of *SMN2* gene increase to approximately 1.5, indicating the presence of 3 copies of *SMN2* (open arrows).

which can lead to a false-negative result when testing for SMA carrier [11]. In this light, the 2+0 genotype is an essential factor that needs to be considered when calculating the risk of being an SMA carrier, the carrier frequency, and the rates of *de novo* mutation. Therefore, the estimation of the frequency of the 3-copy *SMN1* genotype in the Korean population is essential for a more reliable assessment of genetic risk. Lee et al. [5] have reported that in a Korean population, the frequency for 3-copy *SMN1* genotype, calculated using real-time PCR, was approximately 19%. Huang et al. [12] conducted a study on a Taiwanese population using MLPA and reported the frequency of 3-copy *SMN1* to be 7.1%. Our study, which used the same assay, yielded similar results in Korean population (*P* value=0.08).

We also assessed the *SMN2* copy numbers in this study population, and observed a high degree of individual variation. Sixty-four percent of the individuals had 2 copies of *SMN2*, but 36% had 0, 1, or 3 copies of this gene. Since a higher copy number of the *SMN2* has an inverse relation with the severity of SMA [2], the accurate identification of *SMN2* copy numbers can serve as a useful tool in predicting the prognosis of patients with SMA. Although the *SMN2* gene number assay would be helpful in a clinical setting, the performance standard for this assay has not yet been established. The most important shortcoming of the *SMN2* assay is the lack of adequate controls for its validation. To determine whether there are any variations in *SMN2* copy numbers, MLPA peak profiles of patients should be compared with those of reference samples with normal copy numbers. For example, if we compare the MLPA peak pattern of an SMA patient with 2 copies of *SMN2* with that of the reference sample with 1 copy of *SMN2*, the *SMN2* copy number in the patient will show a false-positive increase. Thus, our results of the *SMN2* genotype distributions will facilitate accurate selection of reference samples with 2 copies of *SMN2* in the Korean population.

In summary, race- and ethnicity-based variation in the carrier frequency and the '2+0' genotype are important factors that should be considered when estimating the risk of being an SMA carrier. In addition, *de novo* mutation rates and intragenic *SMN* mutations have to be evaluated. The SMA carrier frequency in the Korean population appears to be 1 in 50, which indicates the prevalence of SMA in Korea is similar to that in the Western countries. The frequency of individuals with the '2+0' genotype was estimated to be 1.0%. In MLPA analysis for determina-

tion of variations in *SMN2* copy numbers, it is important to use appropriate reference samples with normal copy numbers.

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