

Possible association between L-selectin gene P213S polymorphism and respiratory complications of childhood spinal muscular atrophy patients

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Abstract

L-selectin is a cell adhesion molecule that plays a very important role in the initial phase of leukocyte – endothelial cells interaction and thus in the inflammatory response. The aim of our study was to investigate the possible relationship between L-selectin gene P213S polymorphism and the respiratory complications of patients affected by childhood spinal muscular atrophy. Sixty patients and sixty healthy subjects were genotyped, by PCR-RFLP analysis. The frequency of 213 S allele was higher in patients group than in the control one (43.3% vs 20%). For the moment our data might lead to the conclusion that 213 S allele of L-selectin gene could confer risk for respiratory complications of childhood spinal muscular atrophy patients, but it is obviously that further studies are required to establish a more accurate estimation of this complex relationship.

Keywords: spinal muscular atrophy, L-selectin gene, polymorphism

Introduction

The childhood form of spinal muscular atrophy (SMA) is a neuromuscular disorder with a worldwide reported frequency of 1:6000 – 1:10000 [1]. The disease is characterized by severe generalized hypotonia and atrophy of proximal muscles, including intercostals and accessory respiratory ones. Bronchopulmonary infections and other respiratory illness were often reported as the cause of death in SMA patients [2]. The role of genetic predisposition in these complications is not well characterized. A possible role was attributed to the NAIP gene (5q12.2-13.3), based on the observation that some patients with deletion of NAIP exon 5 have a more rapid deterioration of respiratory function [3]. Although this role was not confirmed by the subsequent studies, the researchers tried to identify other possible genetic markers involved in these complications [4].

L-selectin is a cell adhesion molecule, involved in leukocyte-endothelial cell interaction and inflammatory cell recruitment [5]. The gene L-selectin (1q23-q25) comprises 10 exons and spans approximately 30 kb. The exons 6 and 7 are coding for the SCR (Short Consensus Repeats) domains that are thought to play important roles in cell adhesion, oligomerization and optimal presentation of EGF and lectin domains [6]. Thus, polymorphisms in these exons may affect the function of the SCR domain and could be associated with different inflammatory diseases.

The aim of this study was to investigate the presumptive relationship between the respiratory complications of SMA disease and genetic variation in L-selectin gene.

Materials and methods

Subjects

One hundred and twenty subjects with Caucasian origin were included in this study. Sixty SMA patients (with age between 2 months and 19 years) from “Al. Obregia” Psychiatry Clinical Hospital, were clinically diagnosed according to the SMA International Consortium criteria with types I, II and III of disease. The clinical diagnosis was confirmed by molecular analysis (detection of deletions in SMN gene exons 7 and 8 and NAIP gene exon 5).

The sex-matched healthy control lot was selected from subjects without neuromuscular diseases or respiratory problems in their families. The informed consent of subjects or legal tutors was obtained according to the Declaration of Helsinki.

DNA isolation

DNA was isolated from 500 µl blood stored on EDTA anticoagulant, using Promega Wizard Isolation kit (Promega Corporation, Madison, USA) and stored at 4°C.

PCR-RFLP analysis

Genotyping of L-selectin gene P213S polymorphism was assessed using PCR-RFLP method. The amplification reaction was performed in a Perkin Elmer Gene Amp PCR system 2400, in 25 µl reaction volume containing approximately 200 ng genomic DNA, 200 µM each of dATP, dCTP, dGTP, dTTP, 50 mM KCl, 10 mM Tris-HCl, 3mM MgCl₂, 0.5 µM of each primer, 1 unit of *Taq* polymerase. The sequences of primers were the same as it were described previously by Kamiuchi: forward 5'-TGATTCAGTGTGAGCCTTTG-3' and reverse 5'-CTTGACAGGTTGGTTCTG-3' [7]. PCR reactions were carried out in the following conditions: 2 min at 94°C, 30 cycles for 1 min at 94°C, 50 sec at 59°C, 40 sec at 72°C and 1 min at 72°C.

The PCR products (186 pb length) were verified by agarose gel (2%) electrophoresis and then digested for three hours with 5U/reaction *Hph* I enzyme (New England Biolabs, Inc. Beverly, USA), according to the manufacturer instructions. The restriction products were visualized in an 8% polyacrilamide gel, under UV light, after ethidium bromide staining.

Statistical analysis

The distribution of genotypes in patients and control lots was first tested for the Hardy-Weinberg equilibrium condition. The chi-square test (χ^2 , with a value of $p < 0.05$ considered statistically significant) was applied to compare the observed genotypes distribution with the expected one. Because the number of SS homozygous genotypes was low in both lots, the Yates correction was applied.

Results and Discussions

Three genotypes for L-selectin gene P213S polymorphism were determined by PCR-RFLP method. Because the presence of 213S allele creates a situs for the *Hph*I digestion enzyme, two fragments of 142 bp and 44 bp have been identified after restriction of amplicons. In the case of 213 P allele, the PCR product is not digested (Figure 1).

Allelic and genotypic frequencies (indicated in Table 1) were calculated after direct genotypes counting in both lots.

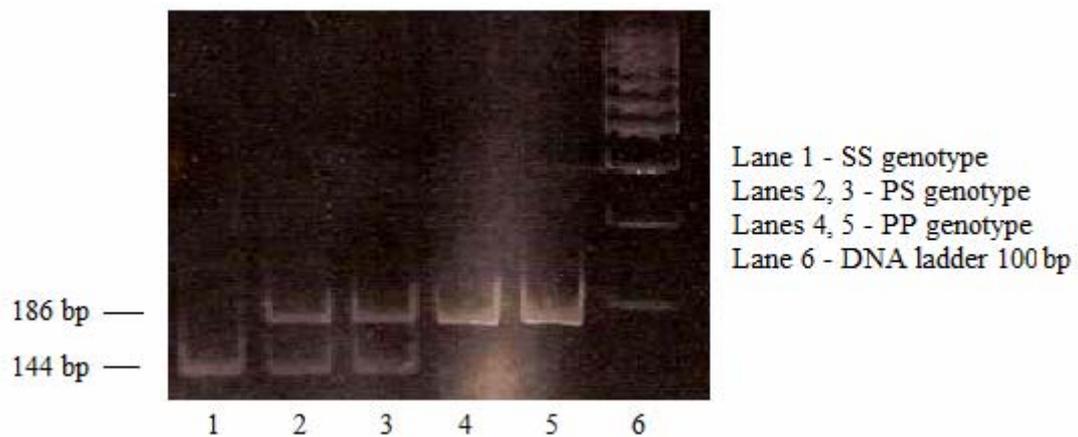


Figure 1. The electrophoresis results of P213S genotyping.

Table 1. The distribution of genotypes and alleles in case and control lots.

Subjects	Genotype			Allele	
	PP	PS	SS	P	S
SMA patients lot	14 (23,3%)	40 (66,7%)	6 (10%)	68 (56,7%)	52 (43,3%)
Healthy subjects lot	39 (65%)	18 (30%)	3 (5%)	96 (80%)	24 (20%)

As no significant sex differences was identified in the genotypes distribution for patients and neither for the healthy subjects groups, the dichotomy males-females was not shown in the table. The χ^2 test performed for each lot revealed that SMA group is not in a Hardy -Weinberg equilibrium ($\chi^2 = 7.66$, $p = 0.005$, $DF = 1$), and that the genotype SS is under represented.

The SS genotype is more frequent in SMA patients than in control group (10% vs 5%). The result $\chi^2_{Yates} = 0.48$ ($DF = 1$, $p = 0.4882$) shows that the genotype SS is not significantly associated with SMA phenotype in the patients. Regarding the S allele, it could be observed that its frequency in patients (43.3%) is two-fold increased in comparison with control lot (20%). In contrast to genotype involvement, the S allele might be a possible risk factor for the respiratory complications in SMA disease, as the statistical results indicates: $OR_S = 3.058$, $CI = 95\%$, $1.7215 < OR < 5.435$.

The association between L-selectin gene P213S polymorphism and different diseases was also investigated in recent studies. A possible (and in some cases, strength) association was reported for the PP genotype and risk for nephropathy in type 2 diabetes mellitus [7], cardiomyopathy [8] or Grave's disease [9]. It was revealed that 213S allele may have a contribution in low-birth weight infants, especially in those with bronchopulmonary dysplasia [10].

After our knowledge, the present study represents the first research regarding the involvement of L-selectin gene polymorphism in respiratory complications of childhood spinal muscular atrophy. The investigated SMA patients lot was not in accordance with the Hardy-Weinberg equilibrium law and the under representation of SS genotype could be observed. Adding to these findings the fact that statistical analysis indicated a possible risk conferred only by the S allele, we think that a plausible explanation could be the natural selection pressure against SS genotype.

However, we cannot exclude the probability that a bias have been occurred in our pilot study and that the results have been affected by at least the effects of population stratification and randomization sampling. Additional researches will be assessed to confirm our results. Also, replicates studies performed in other populations will be useful for the interpretation of our findings.

Conclusion

Our preliminary results suggest a possible association between L-selectin P213S polymorphism and respiratory complications of childhood SMA patients, despite all the possible inconveniences.

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