INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive late-onset neurodegenerative disease. It is clinically characterized by the selective death of motor neurons in the motor cortex, brainstem and spinal cord. Ten percent of ALS cases are familial (FALS), sporadic ALS (SALS) comprising 90% per cent of all patients. The major locus, corresponding to 20 per cent of all FALS cases, is on chromosome 21q, coding for superoxide dismutase 1 (SOD1). It spans 12 kb of DNA and has five exons and four introns. In SALS pathogenesis, genes and environmental factors are in complex interactions. Recently, the vascular endothelial growth factor (VEGF) gene has been implicated in ALS as a ‘modifier gene’, associated with motor neuron degeneration. It was shown that homozygosity for specific VEGF promoter haplotype SNP haplotypes (~2578A→-1154A→-634G or ~2578A→-1154G→-634G) results in 1.8 times greater risk for developing ALS. Angiogenin (ANG), another modifying gene in ALS, is similar in function to VEGF. An increase in the frequency of the G allele of the rs11701 SNP and seven missense mutations in the ANG gene have been reported in the ALS population.

In the present study, the possible sequence alterations in the SOD1 gene were investigated among 153 ALS patients, while 101 ALS patients and 99 healthy controls were screened for the SNPs in the VEGF and ANG genes.

METHODS

RESULTS & DISCUSSION

A) SOD1 Gene Analysis:

DNA sequencing of the SOD1 gene revealed A → C transversion in three patients (Figure 1) which was reported to be a rare polymorphism with a frequency of 4% (Deng, H.X., et al., 1993)

Additionally, one patient showed G → C transversion in exon 5 position 144 (Figure 2).

Until today, two per cent of FALS cases and only a few SALS patients were reported to exhibit a SOD1 mutation. Also, an apparent heterogeneity in the distribution of SOD1 mutations in different ethnic groups have been shown in several studies. In this respect, when both the low percentage and heterogeneity hypothesis are considered, the lack of SOD1 gene mutations in our study population can be reasoned.

B) VEGF Gene Analysis:

Taking together all the SNP results at positions ~2578A/C, ~1154A/G and ~634C/G, individuals homozygous for the haplotypes (~2578A, ~1154A, ~634G) or (~2578A, ~1154G, ~634G) were collected under the name of “at-risk genotypes”, and all the other remaining genotype possibilities were considered as “not-at-risk genotypes” (Figure 3).

The distribution of the VEGF at-risk genotypes were found to be statistically significant in our study population (G²=5.34, df=2, p=0.08). The transition of the G nucleotide to A results in the replacement of the originally encoded amino acid valine by isoleucine on the peptide chain at position 103 (Figure 5).

Neither the genotype, nor the allele distributions of rs11701 SNP was found to be statistically significant in our study population (G²=0.51, df=1, p=1).

After completing the rs11701 SNP genotyping, the presence of further sequence alterations in the ANG coding sequence was investigated. A heterozygous presence of a possible novel lesion, a G to A transition, was identified, which was previously not reported. The transition of the G nucleotide to A results in the replacement of the originally encoded amino acid valine by isoleucine on the peptide chain at position 103 (Figure 5).

This is a preliminary result, which has to be verified by further analysis of the patient and his family.

In conclusion, the previously reported associations between ALS and the genetic variants of VEGF and ANG, which confer a risk for the development of the disease, were not observed in our study population. However, this lack of association does not rule out the role of these genes in ALS generally. It is highly possible that these different results may be due to the different genetic backgrounds of individuals from different countries, investigated. Regarding the complexity and heterogeneity of ALS, it should also be taken in consideration that the differential diagnosis of patients may not have been correct in single cases, which would lead to false negative associations between ALS and the disease-related genes.

REFERENCES

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