Polymorphisms in FAS and CASP8 genes may contribute to the development of ALPS phenotype: a study in 25 patients with probable ALPS

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Defects in genes that have role in apoptotic pathways result in development of Autoimmune Lymphoproliferative Syndrome (ALPS) and ALPS related disorders. Germline and somatic FAS mutations, FASL and CASP10 mutations constitute other genetic defects in ALPS. Patients who fulfill ALPS diagnostic criteria and do not have any identified known disease causing mutations are classified as ALPS-unknown or ALPS phenotype and comprise about one third of all patients. CASP8, NRAS and KRAS gene mutations were reported for ALPS related diseases. We performed DNA sequence analysis in 25 unrelated patients with probable ALPS for FAS, FASL and CASP8 gene defects. Pathogenic mutations could not be found in the FAS, FASL and CASP8 genes. However, we found that the frequencies of SNPs rs2234978 and rs1045487 of FAS and CASP8 genes were significantly higher in the patients. Our results suggest that CASP8 and FAS gene polymorphisms in particular, may contribute to the susceptibility to development of ALPS phenotype.

Key words: ALPS phenotype, FAS, FASL, CASP8, SNP

Apoptosis plays an important role in the termination of immune response, and is important in central immune tolerance and maintenance of peripheral tolerance¹,². Defects in genes that have role in apoptotic pathways result in dysregulation of lymphocyte homeostasis and development of ALPS and ALPS related disorders. Autoimmune lymphoproliferative disorder is characterized by chronic non-infectious, non-malignant lymphadenopathy and/or splenomegaly, autoimmune features, most commonly autoimmune cytopenias, increased numbers of Double Negative T (DNT) cells in peripheral blood and susceptibility to development of lymphoma³.

Most patients with ALPS are associated with germline heterozygous mutations of the FAS gene³. Somatic FAS mutations constitute the second most common genetic defect for ALPS development³. In a small number of patients FASL and CASP10 gene defects are responsible for ALPS: Patients who fulfill ALPS diagnostic criteria in whom no mutations can be identified are classified as ALPS-unknown (ALPS-U) or ALPS phenotype and comprise about one third of all patients³,⁴. CASP8, NRAS and KRAS mutations were reported for ALPS related diseases³. In this study we performed DNA sequence analysis in 25 unrelated patients diagnosed as probable ALPS according to the criteria revised in 2009 to detect whether they have underlying FAS, FASL and CASP8 gene defects³.

Material and Methods

The patients included in the study were diagnosed at and followed by Hacettepe University Pediatric Immunology division. Twenty five patients (17 boys, 8 girls) with probable ALPS, diagnosed according to the criteria revised in 2009³ and 100 healthy controls were enrolled in the study. Genomic DNA from the peripheral blood was isolated by using EZ1 DNA Blood 200 ul Kit (Qiagen). Written consent was obtained prior to sampling

All coding exonic fragments and the exon-intron boundaries of the FAS, FASL and CASP8 genes were amplified and sequenced for detection of mutations. The primer sequences are available upon request. The polymerase chain reaction (PCR) products were cycle sequenced on Applied Biosystems 310 genetic analyzer using ABI Big-Dye 3.1 reagents according to the manufacturer’s recommendations.

The percentages of peripheral blood DNT cells, T lymphocyte subpopulations, immunoglobulin (Ig) values, antibody responses (against rubella, varicella, HBs Ag, HAV, HSV, EBV and pneumococcal polysaccharides), total lymphocyte and neutrophil counts were obtained from patients’ charts. The differences in frequencies of SNPs between patients and healthy controls were analyzed by Fischer’s Exact Chi Square test.

**Results**

The percentages of peripheral blood DNT cells were found to be high (>1.5% of total lymphocytes or >2.5% of T lymphocytes) in all patients. The values were between 2-13% with median value of 3%.

No disease causing pathogenic alleles were found in the FAS gene, however nucleotide changes; rs2234978 C>T was present in both patients and controls. The homozygous T allele (minor allele) was detected in 22 patients (88%), one patient was heterozygous for this

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number and percentage of patients</th>
<th>Number and percentage of controls</th>
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<tbody>
<tr>
<td>C/C</td>
<td>2 (8%)</td>
<td>66 (73%)</td>
</tr>
<tr>
<td>C/T</td>
<td>1 (4%)</td>
<td>22 (25%)</td>
</tr>
<tr>
<td>T/T</td>
<td>22 (88%)</td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

**Table I.** The Frequency of FAS SNP rs2234978 C/T in patients (n=25) and controls (n=90).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number and percentage of patients</th>
<th>Number and percentage of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/A</td>
<td>5 (20%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>G/G</td>
<td>20 (80%)</td>
<td>98 (98%)</td>
</tr>
<tr>
<td>A/A</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table II.** The Frequency of CASP8 SNP rs1045487 G/A in patients (n=25) and controls (n=100).

![A) rs 2234978 (wild type)](image)

![B) rs 2234978 Minor allele heterozygous T (c/T)](image)

![C) rs 2234978 Minor allele homozygous T](image)

![Fig. 1. Examples of FAS Gene SNP rs2234978 (C>T)](image)
allele (4%). These values were 2 (2%) and 22 (25%) for controls respectively (Table I, Fig. 1A-C). The difference between the frequency of minor allele T in patients (92%) and controls (27%) was found to be statistically significant (Fischer’s Exact Chi-Square test p=0.001), (OR=0, 0188, 95% CI=0, 0068-0, 0517).

No mutation or variation could be found in FASL in patients or controls. Mutations were also not present in the CASP8 gene however rs1045487 (G>A), minor allele A, was present heterozygously in 5 of 25 (20%) patients and in 2 of 100 (2%) controls. Homozygous allele A was not detected in patients or control group (Table II, Fig. 2A-B). The frequency of G/G and G/A genotypes were statistically different in patients than control group (Fischer’s Exact Chi-Square test p=0.004), (OR=0, 0909, 95% CI=0, 0171-0, 04836).

Discussion

This study was done in 25 patients diagnosed as probable ALPS. Patients had non-infectious, non-malignant lymphadenopathy and/or splenomegaly lasted longer than 6 months, increased percentage of DNT cells and autoimmune cytopenias which comprise secondary accessory criteria for the diagnosis of ALPS3.

We detected only SNP rs2234978 and rs1045487 in FAS and CASP8 genes respectively in both patient and control groups. No mutation or variation was found in FASL in both groups. The frequencies of SNP rs2234978 of FAS gene (homozygous minor T allele) and rs1045487 of CASP8 (heterozygous minor A allele) significantly increased in our patients as compared to healthy controls (p=0.001 and p=0.004 respectively). Our results suggest that CASP8 and FAS gene polymorphisms in particular, may contribute to the susceptibility to the development of ALPS phenotype. Whether these SNPs have any effect, either with affecting the expression of FAS itself or via linkage to other genetic abnormalities playing role in the pathogenesis of the disease needs to be determined. In single gene disorders, SNPs present in other genes may contribute to the development or to the phenotypic features of the diseases. Most SNPs are silent and do not have a demonstrative effect on the gene function or phenotype of the disease. However, some variations in the transcription factor binding site (e.g. in promoter regions) or other regulatory regions may affect the expression level of the gene5,6. It has been shown that rs2234978 polymorphism created a functional binding site for miRNA (Mir-561). miRNAs contribute to regulation of protein synthesis by changing the expression of target genes 7. In fact the haplotypes that create susceptibility to acute lung injury (ALI) which included SNP rs2234978 have been shown to increase the expression of FAS mRNA in peripheral blood leukocytes stimulated by lipopolysaccharides 8.

Studies indicate that dysfunction in apoptosis may have a role in the pathogenesis of autoimmune disorders like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), systemic sclerosis (SSc) and multiple sclerosis (MS) 9-11. Various studies done in different
populations showed that there is an association between FAS gene polymorphisms in promoter region in particular and with the risk of SLE and MS\(^9\). Associations between the SNP rs2234978 and presence of thrombocytopenia in SLE patients have also been demonstrated\(^10\). In addition, studies in animal models of SLE showed inbred genetic background affects disease expression in lpr or gold murine counterparts and the mutations in the FAS and FASL is not sufficient leading to the development of SLE but contribute to the autoimmunity\(^11\).

The heterozygous germline FAS mutations are responsible for most of the ALPS patients. However, the disease has variable penetrance and severity and family members with the same mutation may be healthy or present with very mild phenotype\(^12\). These observations suggest a contribution of a second hit (like an additional genetic mutation and/or environmental stimulation) for the development of the disease\(^3\). Examples include a patient with both FAS and Perforin mutations whose parents carried one of these mutations were healthy\(^14\); and other patient with both FAS mutation and CASP10 variation whose mother with the same FAS mutation was healthy\(^15\). In another family while the patients who carried both paternally inherited c.761T>G missense mutation and maternally inherited c.642C>T SNP in FAS gene were severely affected, the father was asymptomatic until age 31 years and the mother was healthy\(^16\). Chatinet et al. demonstrated that the variable penetrance of the disease may be explained by the presence of multiple FAS mutations\(^17\). These investigators reported 7 patients who had heterozygous FAS mutations and developed somatic variations in FAS. The individuals of these families who carried only the same germline FAS mutation have been asymptomatic. Although multiple abnormalities in FAS gene may explain the variable penetrance, mutations acquired in other genes may also be present\(^17\). Six different variations in UNC13D gene have been demonstrated in patients with ALPS/ FAS, ALPS-U and Dianzani Autoimmune Lymphoproliferative Disease (DALD) in 29%, 21% and 15% of respective patients\(^18\).

We could not find any mutations other than SNPs in FAS and CASP8 genes, however mutations in some other genes in association with FAS and/or CASP8 gene SNPs may lead to ALPS phenotype in our patients. Among our patients with homozygous minor allele rs2234978 in FAS, four had also carried rs1045487 in CASP8 gene. Clinical and laboratory features did not differ between patients carrying both variants or carrying FAS variant only.

Rensing et al\(^19\) proposed that patients with features compatible with CVID associating autoimmune cytopenias are needed to be investigated for ALPS\(^19\). In fact, although hypergammaglobulinemia is among the secondary criterion for diagnosis of ALPS, there are many patients with decreased serum Ig isotypes in various combinations and a significant overlap between ALPS and CVID has been reported\(^19\). One or more Ig isotypes in various combinations were decreased in about half of our patients. However most of these values were mildly low and some were not persistently low during follow-up period. Five patients showed progressive decline in Ig isotypes, 3 of which were in IgG. Antibody responses (against rubella, varicella, HBsAg, HAV, HSV, EBV and pneumococcal polysaccharides) were positive at least against one half of the antigens studied. With the results of Ig levels and antibody production, 5 of our patients were considered as having “probable” common variable immunodeficiency (CVID)\(^20\).

Published data have demonstrated important immunologic and clinical overlap also between ALPS and Evans Syndrome and DALD\(^13\). The underlying molecular defects in many of these patients have not yet been discovered. Evans Syndrome (ES) is a hematologic disorder, defined by two or more autoimmune cytopenias. Either idiopathic thrombocytopenic purpura (ITP) or AIHA was present in the majority of our patients and both were present in two. A significant percentages of patients diagnosed as ES may have ALPS. In two studies, it has been demonstrated that 6 out of 12 patients\(^21\) and 21 out of 45 patients with ES\(^22\) carried diagnostic criteria of ALPS.

In conclusion, our results suggest that CASP8 and FAS gene polymorphism in particular, may contribute to the susceptibility to the development of ALPS phenotype. Whether these
SNPs particularly rs2234978 of FAS may be causal to the development of ALPS phenotype either via changing the gene expression or via linkage to other genetic abnormalities needs to be determined.

REFERENCES