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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SUMMARY: Tan Ç, Özgül RK, Çağdaş-Ayvaz D, Tezcan İ, Sanal Ö. Polymorphisms in *FAS* and *CASP8* genes may contribute to the development of ALPS phenotype: a study in 25 patients with probable ALPS. Turk J Pediatr 2015; 57: 141-145.

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 tolerance[1,2]. 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 lymphodenopathy 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 lymphoma[3].

Most patients with ALPS are associated with germline heterozygous mutations of the *FAS* gene[3]. Somatic *FAS* mutations constitute the second most common genetic defect for ALPS development[3]. 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 patients[3,4]. *CASP8*, *NRAS* and *KRAS* mutations were reported for ALPS related diseases[3] . 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 defects[3].

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 2009[3] 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

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

Phenotype	Number and percentage of patients	Number and percentage of controls
C/C	2 (8%)	66 (73%)
C/T	1 (4%)	22 (25%)
T/T	22 (88%)	2 (2%)

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

Phenotype	Number and percentage of patients	Number and percentage of controls
G/A	5 (20%)	2 (2%)
G/G	20 (80%)	98 (98%)
A/A	0	0

in all participants. Ethics Committee ID: FUND 11 / 19-23. Supported by H.U.B.A.B (FON11/19).

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.

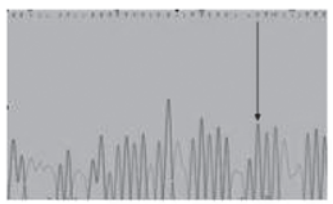
The percentages of DNT cells, T lymphocyte subpopulations, immunoglobulin (Ig) values, antibody responses (against rubella, varicella, HBs Ag, HAV, HSV, EBV and pneumococcal polysaccharides), total lymphocyte and neutrophil counts were obtained from patients' charts. The differences in frequencies of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

Results

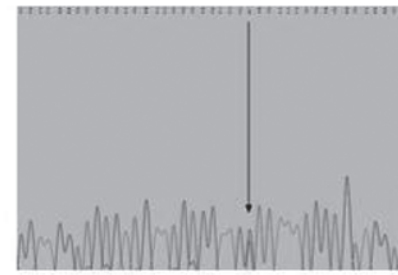
The percentages of peripheral blood DNT cells were found to be high (>1.5% of total lymphocytes or >2.5% of T lymphocytes) in all patients. The values were between 2-13% with median value of 3%.

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

A) rs 2234978 (wild type)



B) rs 2234978 Minor allele heterozygous T (c/T)



C) rs 2234978 Minor allele homozygous T

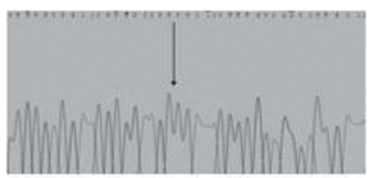
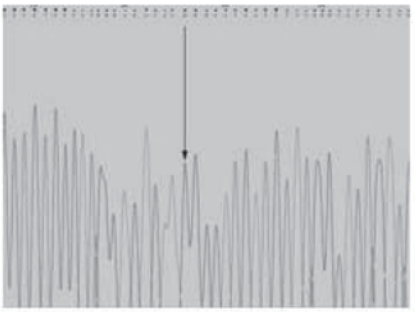


Fig. 1. Examples of FAS Gene SNP rs2234978 (C>T)

A) rs1045487 (G/G) (wild type)



B) rs1045487 Minor allele heterozygous A (G/A)

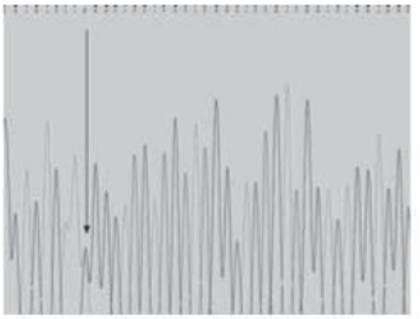


Fig. 2. Examples of CASP8 Gene SNP rs1045487 (G>A)

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 ALPS[3] .

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 C*ASP8* 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 gene[5,6] . 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 erythematosis (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.

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