

The Association of PRKRAP1 Pseudogene with Acute Lymphoblastic Leukemia Risk

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Abstract

Objective: Acute Lymphoblastic Leukemia (ALL) consists of excessive proliferation of lymphoblasts. The frequency of Human Leukocyte Antigen (HLA)-DR53 (DRB4) homozygosity and allele was found to be higher in male childhood ALL cases. The aim of this study was to identify the HLA-DR53 allele frequency; interferon-inducing double chain ribonucleic acid binding protein kinase A pseudogene 1 (PRKRAP1) positivity; rs2395185 allele and genotype frequencies in ALL patients.

Materials and Methods: Sixty ALL patients and 40 healthy controls were studied. HLA's were analyzed using the PCR-SSP. The PRKRAP1 positivity have been identified by PCR and rs2395185 genotypes were determined by TaqMan assay using real-time PCR.

Results: PRKRAP1 positivity was shown to be specific to HLA-DRB4 haplotype in the whole group. We observed that HLA-DRB1*04, DRB1*07 alleles were higher in male patients (43.5%, 25.0%) compared with female patients (25.0%, 21.4%). The prevalence of rs2395185 T allele, HLA-DRB4 allele and HLA-DRB4 homozygous in childhood ALL patients were significantly higher in males compared with the females (p:0.044, p:0.007, p:0.045, respectively).

Conclusion: The molecular mechanism of PRKRAP1 pseudogenesis, which we have confirmed to be specific for HLA-DRB4 (DR53) haplotypes, in a newly identified specific transcription map can be investigated and its relationship with ALL can be determined.

Keywords: Acute lymphoblastic leukemia, HLA-DR53, HLA-DRB4, PRKRAP1, Genetic polymorphism, Genotype frequencies, Pseudogenesis

Introduction and Aim

Acute lymphoblastic leukemia (ALL) is the deterioration of the maturation period of lymphoblasts, and the uncontrolled excessive reproduction of the abnormal cells. In addition to gene rearrangements, chromosome number abnormalities (Down syndrome, etc.), and genetic locations, being male, Caucasian ethnicity, having a sibling diagnosed with leukemia, exposure to radiation, having received chemotherapy or radiotherapy, and exposure to some toxins and chemical substances such as benzene may be counted as the ALL risk factors.

The Human Leukocyte Antigen (HLA) genes that were

shown to be associated with various immune diseases are localised on the short arm (6p21) of the chromosome 6. The individuals carrying the HLA-DRB1*04, HLA-DRB1*07 or HLA-DRB1*09 alleles which are encoded by the HLA Class II genes in this gene group also carry the HLA-DRB4 (HLA-DR53) haplotype [1]. The frequency of HLA-DRB4, and HLA-DRB4*01 homozygous was found to be higher specific to boys in childhood ALL in other studies associated with the HLA-DR haplotypes [2,3].

rs2395185 is located in the intergenic region of the HLA-DR sub-region (between the HLA-DRA and -DQA1 genes). Thymine (T) allele variant is the indicator of HLA-DRB4 haplotype [1]. rs2395185 was found to be protective

against Hodgkin's lymphoma; and a risk factor for ALL, lung cancer, rheumatoid arthritis, and asthma in previous studies [4-8].

HLA-DRB4 haplotypes are rich for pseudogenes whose biologic roles have not yet been completely understood pseudogenes were found located in HLA-DRB4 region, located between HLA-DRA and HLA-DQA1 [9]. *PRKRA* (Protein Kinase, Interferon Inducible Double Stranded Ribonucleic Acid Dependent Activator; *PRKRA*) gene is located in this region, and was suggested to be a pseudogene owing to not including an exon [10]. The pseudogenes specific to HLA-DRB4 family was suggested to be able to transcribe after demonstrating that the pseudogenes in HLA-DRB4 region had a 70% transcription rate [9-11].

The aim of the present study was to identify the HLA-DRB4 frequency in ALL patients, and to compare the *PRKRA* positivity and rs2395185 variant allele (thymine) positivity with the HLA-DRB4 allele frequency.

Material and Method

Patient, and control group

Sixty ALL patients below 18 years whose HLA typing were studied for hemopoietic stem cell transplant, and 40 healthy individuals over 18 years with no consanguineous relationship with one another between 2008 and 2015 were included in the study. 46 were male (76.7%), and the age interval was between 1-17 years (7.79 ± 5.1 years), and 14 were female (23.3%), and the age interval was between 1-17 years (7.93 ± 4.8 years) in the patient group. 19 were male (47.5%), and the age interval was between 21-41 years (29.11 ± 5.5 years), and 21 were female (52.5%), and the age interval was between 21-41 years (28.95 ± 5.5 years) in the control group.

DNA isolation

DNA isolations were performed using the salting out method after peripheral blood samples were drawn from patients and healthy individuals into 7 mL EDTA tubes. The samples were stored at -20°C before use.

HLA Typing

The HLA typings (HLA-DRB1/3/4/5 loci) of the patients and healthy individuals included in the study were performed at low resolution level using the polymerase chain reaction- Sequence Specific Primer (PCR-SSP) (Olerup) method.

PRKRAP1 presence/absence determination

The DNA products obtained by PCR using the primers specific to internal control sequence (*TAPA*) together with

PRKRAP1 gene were screened under ultraviolet light using the 1.5% agarose gel electrophoresis.

The her6l (5'-TTGGTTCATTACAGGAATTGG-3'), and her3r(5'CAAAGAAGAGCTTAAGCACC-3') oligonucleotides specific to *PRKRAP1* gene were synthesized [10,12]. We aimed to reproduce the region of 1.2 kb at chromosome 6 using the PCR with the use of the her6l and her3r main primers. The 5'-TCATGCCCTCCCAACCC-3' and 3'-CCCACCCTCTACCCCTG-5' primers of *TAPA* were also purchased as the internal control primer together with the main primers.

rs2395185 typing

10 μL precision@FAST2X Q-PCR master mix, 1 μL rs2395185 primer/TaqMan probe mix, and 4 μL RNase/DNase free water were used for the real time PCR genotyping reaction mix component content. 15 μL was added to the 0.1 mL PCR reaction tubes from the Q-PCR experiment plate well, and the final volume in all wells were completed to 20 μL with the inclusion of 5 μL DNA sample with a DNA concentration of 0.2-2ng/ μL (1-10ng in total). The fluorescent readable Rotor Gene Q (Qiagen) was used as the real time PCR device using the VIC and ROX channels. The amplification protocol was arranged as the enzyme activation for 2 min at 95°C ; 10 cycle lasting 10 seconds denaturation at 95°C , elongation for 60 seconds at 60°C , and 50 cycles of 10 seconds denaturation at 95°C , and elongation (data collection) for 60 seconds at 68°C .

Statistical methods

The statistical analysis of the obtained data were performed using the Statistical Package for the Social Sciences (SPSS 21.0) software program. Descriptive analyses, and descriptive statistical methods were used. The Chi-square test was used for identifying the statistical significance of the differences between the counts in groups. The Student's t test was used in the numerical comparisons between two groups with normal distribution, and Mann-Whitney test was used in the comparison of two groups with nonnormal distribution. Kruskal-Wallis test was used in the numerical comparisons in non-normal distribution data, and in the comparison of multiple groups.

Results

No statistical difference was detected between the patient and control groups for HLA-DRB1 alleles ($p>0.05$). DRB1*04, DBA1*07, and DRB1*11 alleles were the most frequently detected alleles in both groups. HLA-DRB1*13 allele was found statistically significantly higher in female patients ($p=0.007$) (Table 1).

DRB4 (DR53) positivity had no statistically significant difference between the patient (83,3%, $n=50$), and control

HLA-DR	ALL Male Patients		ALL Female Patients		p	OR (%95 CI)	Control Group	
	n=46	Frequency	n=14	Frequency			n=40	Frequency
DRB1*01	0	(0.0%)	0	(0.0%)	-	-	1	(1.3%)
DRB1*03	4	(4.3%)	0	(0.0%)	0.572	2.898 (0.151-55.531)	1	(1.3%)
DRB1*04	40	(43.5%)	7	(25.0%)	0.120	2.308 (0.892-5.966)	38	(47.5%)
DRB1*07	23	(25.0%)	6	(21.4%)	0.804	1.222 (0.441-3.386)	12	(15.0%)
DRB1*08	0	(0.0%)	0	(0.0%)	-	-	0	(0.0%)
DRB1*09	2	(2.2%)	0	(0.0%)	0.999	1.339 (0.119-15.026)	1	(1.3%)
DRB1*10	0	(0.0%)	0	(0.0%)	-	-	0	(0.0%)
DRB1*11	8	(8.7%)	5	(17.9%)	0.179	0.438 (0.130-1.468)	8	(10.0%)
DRB1*12	0	(0.0%)	0	(0.0%)	-	-	0	(0.0%)
DRB1*13	2	(2.2%)	5	(17.9%)	0.007	0.102 (0.018-0.561)	4	(5.0%)
DRB1*14	0	(0.0%)	2	(7.1%)	0.999	1.575 (0.073-33.790)	2	(2.5%)
DRB1*15	7	(7.6%)	0	(0.0%)	0.198	5.000 (0.276-90.386)	6	(7.5%)
DRB1*16	6	(6.5%)	3	(10.7%)	0.434	0.581 (0.135-2.494)	6	(7.5%)

Table 1: Distribution of HLA-DRB1 alleles.

Patients (n=60)	DR53 (+)		DR53 (-)		PRKRA (+)		PRKRA (-)		rs2395185 T allele (+)		rs2395185 T allele (-)		P
	n	%	n	%	n	%	N	%	n	%	n	%	
Male (n=46)	41	89.1	5	10.9	41	89.1	5	10.9	41	89.1	5	89.1	0.044
Female (n=14)	9	64.3	5	35.7	9	64.3	5	35.7	9	64.3	5	64.3	

Table 2: Presence/absence of DR53, PRKRA and rs2395185 T allele in patients.

groups (72,5%, n=29), however, DRB4 positivity was significantly higher in male patients (p=0.044) (Table 2). Particularly the DRB4 (DR53) homozygosity was statistically higher in male patients (37%, n=17) compared with female patients (7,1% n=1) (p=0.045). There was no statistically significant difference in DRB4 homozygosity rates between the male and female individuals in the control group (p>0.05).

PRKRAP1 pseudogene positivity in the whole sample was specific to HLA-DRB4 haplotype. All samples positive for *PRKRAP1* (n=79) were also positive for DRB4. *PRKRAP1* gene positivity was detected in only DR53 haplotype carrier individuals in both patient, and control groups, however the gene positivity was not detected in individuals with no DR53 haplotype carrier. *PRKRAP1* gene positivity was

detected higher in male patients compared with female patients (p=0.044), and no significance was detected between sexes in the control group (p>0.05) (Table 2).

Variant rs2395185 homozygosity (TT) was detected in HLA-DR53 homozygous (n_{patient}=28, n_{control}=22) and heterozygous (n_{patient}=22, n_{control}=7) samples, however wild-type rs2395185 homozygous (GG) was detected in samples which involved no HLA-DR53 in the study (n_{patient}=10, n_{control}=11). Wild/variant type rs2395185 heterozygous (GT) was detected in HLA-DR53/51, and HLA-DR53/52 heterozygous samples. rs2395185 GT genotype was found significantly higher in the patient group compared with the control group (p=0.045) (Table 3). T allele was found significant in male patients compared with the female patients (p=0.044) (Table 2).

SNP rs2395185	Patients (n=60)		Controls (n=40)		p
	n	%	n	%	
Variant homozygous (TT)	28	46.7	22	55	0.540
Variant/wild type heterozygous (TG)	22	36.7	7	17.5	0.045
Wild Type homozygous (GG)	10	16.6	11	27.5	0.217

Table 3: Comparison of rs2395185 genotypes between patients and controls.

Discussion

HLA Class II genes are the highly polymorphic gene regions [13,14]. The HLA alleles encoded by HLA genes may function as protective or as a risk factor in immune diseases.

HLA-DRB1*04 allele was detected to be specifically higher in childhood ALL [3]. HLA-DRB3 haplotype (especially HLA-DRB1*12 and HLA-DRB1*13 alleles) was less frequently detected in patients. HLA-DRB3 homozygosity was detected to be a protective factor in childhood ALL [2]. In the present study, HLA-DRB1*04 allele frequency showed no significant difference in the patient group compared with the control group, and in males compared with females in the study.

Relationships between HLA class II alleles and childhood leukemia have been reported in a number of studies. Malcolm Taylor et al. It has been reported that this association may be due to an imbalance of HLA allele-restricted peptide binding and T cell activation in the pathogenesis of childhood leukemia, or its binding to an MHC-dependent “leukemia gene” [15]. HLA-DRB1*07, a member of the DRB4 haplotypic group, is associated with childhood ALL and Burkitt’s lymphoma [16]. Some studies in literature reported that in four different populations, germ-cell testis tumors have shown associations with HLA-DRB1*07 [17-19] or *04 [20] both of which belong to the HLA-DRB4 family. Dorak et al. reported that a striking feature of the DRB4 relationship in childhood ALL is strong male specificity [3]. And also in some studies in the literature, a similar gender effect has been reported in different genes such as NQO1*2 and del GSTT for children with ALL [21].

The HLA-DRB4 frequency was found significantly higher in male patients compared with the female patients in our study. HLA-DRB4 homozygous was found significantly higher in male patients compared with the female patients. Our study supports the studies which showed that HLA-DRB4 homozygosity was a risk factor for childhood ALL in males.

The HLA-DRB4 carrying haplotypes were found to have uncharacterised additional DNA of 160kb in the

comparison with the HLA-DRB3 carrying haplotypes in the major histocompatibility complex gene maps [13,22,23]. Newly described pseudogenes whose biologic roles has yet not been completely discovered were detected located on the HLA-DRB4 region between HLA-DRA, and HLA-DQA1 [9]. *PRKRAP1* was accepted as a pseudogene as not having an exon sequence, and was suggested to be a residual inactive gene in the HLA Class II region [10,12]. Pseudogenes were suggested to be nonfunctional old gene residues, however the transcription map studies showed that pseudogenes had a high rates of transcription as 70% in the PGF cell line of the HLA-DRB5 family. The results obtained from this study suggested that the pseudogenes specific to HLA-DRB4 family may also be transcribed [9,11]. The active copy of the *PRKRA* gene is localised on chromosome 2 (2q31.2).

It was observed in individuals who carry the homozygotic or heterozygotic HLA-DRB1*04, HLA-DRB1*07 and HLA-DRB1*09 alleles of *PRKRAP1* pseudogene bant in both patients and control groups or in individuals who carry these alleles with the heterozygotic other HLA-DRB1 alleles in our study. The absence of band in individuals having no HLA-DR53 haplotype showed that *PRKRAP1* pseudogene was carried specific to HLA-DR53, and specific to HLA-DRB1*04, HLA-DRB1*07, and HLA-DRB1*09 alleles.

Data from DbSNP for rs2395185 located in the region of HLA-DRA (chr6: 32433167) shows alternative versions of alleles in the FWD (plus) helix, G / T. 29% of people have the variant allele T. Studies have determined that rs2395185, which is positively and negatively associated with many diseases, is a strong risk factor specific to men only as an HLA-DRB4 marker in childhood ALL [5,24]. We found rs2395185 variant homozygous (TT) in HLA-DRB4 homozygous or HLA-DRB4 heterozygous samples which jointly carried the alleles HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*09. rs2395185 variant/wild type heterozygosity (TG) was detected in samples carrying one of the alleles of HLA-DRB1*04, HLA-DRB1*07, and HLA-DRB1*09. rs2395185 wild type homozygosity (GG) was found in all samples which carried no HLA-DR53 haplotypes. Our study was not designed to re-examine the previously reported HLA-DRB4/rs2385185 association in childhood ALL, but similar results were obtained in our small sample.

We anticipate that the additional DNA of 160 kb in HLA-DRB4 haplotypes whose characterisation has yet not been performed might have a role in the basis of the association of ALL that is associated with HLA-DRB4. We suggest that the complete sequencing of HLA-DRB4 haplotypes, and mapping of the pseudogene-specific transcription of the region in future studies may provide an insight into the possible molecular mechanism of HLA-DRB4 association with ALL.

In Several studies , is shown that PRKRA (interferon-inducible double-stranded RNA-dependent protein kinase activator A) is a protective protein which regulates the adaptation of cells to ER stress and virus-stimulated signaling pathways by activating PKR. There is a hypothetical notion that PKRA and HLA DRB4 alleles will contribute to the pathogenesis of virally derived ALL [25-27].

Conclusion

HLA-DRB4 allele, and HLA-DRB4 homozygosity was found significantly higher in childhood ALL patients in males, and *PRKRAP1* pseudogene presence showed a strong correlation with HLA-DRB4 haplotypes.

Ethics Committee Approval

This study was approved by the ethics committee of the Istanbul University Faculty of Medicine (Ethics no: 2016/347-11/03/2016/05).

Conflict of Interest Statement

The authors of this paper have no conflicts of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation data collection and analysis were performed by Cagla Yavuz, Hayriye Senturk Ciftci, and Demet Kivanc. Lab work was performed by Cagla Yavuz, Literature search was performed by Cagla Yavuz. The first draft of the manuscript was written by Cagla Yavuz, Fatma Savran Oguz, and Cigdem Cinar Kekik. Critically Reviewed and edited by Fatma Savran Oguz, Zeynep Karakas, M. Tevfik Dorak, Cigdem Cinar Kekik, and Hayriye Senturk Ciftci. All authors read and approved the final manuscript.

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