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BRIEF COMMUNICATION

Polymorphisms in genes of the BAFF/APRIL system may constitute risk factors of B-CLL – a preliminary study on a Polish population

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Abstract

The association of single-nucleotide polymorphisms (SNPs) of B-cell activating factor (BAFF)/a proliferation-inducing ligand (APRIL) system with B-cell chronic lymphocytic leukemia (B-CLL) have been suggested, therefore, we investigated 20 SNPs of *BAFF*, *APRIL*, *BAFF-R*, transmembrane activator and calcium modulator and cyclophilin-ligand interactor (*TACI*), B-cell maturation antigen (*BCMA*) genes and the risk and outcome of B-CLL in 187 patients and 296 healthy subjects as well as ligand-receptor gene × gene interactions. Although the obtained *P*-values for all 20 SNPs did not reach statistical significance for this study ($\alpha = 0.003$), the high value of the global chi-squared statistic ($\chi^2_{df=38} = 52.65$; $P = 0.0586$), and obtained values of odds ratio indicate that rs9514828 (*BAFF*), rs3803800 (*APRIL*) and rs4985726 (*TACI*) may be associated with the risk of B-CLL. We observed that the B-CLL patients with the genotype rs9514828CT/rs11570136AA were diagnosed with the disease 12 years later than the whole group of patients in this study.

Aberrant expression of B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) has been observed in non-Hodgkin lymphomas (NHLs) and autoimmune diseases such as Sjorgen's syndrome, rheumatoid arthritis and systemic lupus erythematous (SLE) (1, 2). It has been also demonstrated that BAFF level correlated significantly with some important B-cell chronic lymphocytic leukemia (B-CLL) clinical prognostic parameters (2). The genetic variants of *BAFF* have been shown to be associated with the risk of primary Sjogren's syndrome (pSS) (3, 4). Moreover, single-nucleotide polymorphisms (SNPs) in *BAFF* and BAFF receptor (*BAFF-R* or BAFF receptor 3 (BR3)] genes have been reported to be associated with NHLs including B-CLL (5–7).

We anticipated that genes of the BAFF/APRIL system may constitute the risk factors of B-CLL. Therefore, we investigated the possible association between the genetic variants in *BAFF*, *APRIL*, *BAFF-R*, transmembrane activator and calcium modulator and cyclophilin-ligand interactor (*TACI*) and B-cell maturation antigen (*BCMA*) genes and the risk of B-CLL, as well as with disease outcome. Taking into consideration the fact that

BAFF, APRIL and their receptors exert their role by interaction with each other; we also examined the potential interactions between these genes.

The study group consisted of 187 patients (83 females and 104 males) diagnosed with B-CLL in the Department of Hematology, Neoplastic Disease and Bone marrow Transplantation of Medical University of Wroclaw. Table 1 contains characteristic of B-CLL patients. Diagnosis was made according to the standard clinical and laboratory criteria in accordance with the recommendation of the National Cancer Institute (8). The control population composed of 296 randomly selected blood donors of Polish Caucasian origin (144 females and 152 males). This study was approved by the Ethics Committee of the Medical University of Wroclaw. Written informed consent was obtained from all participants.

Based on available literature and *in silico* analysis of the transcripts of the genes of interest together with 5000 bp upstream and 3000 bp downstream regions we selected 20 SNPs. SNPinfo (9) was used for TagSNPs selection and both SNPinfo and FastSNP were used for SNPs function prediction (9, 10). Tables S1–S5 (Supporting Information) contain all SNPs investigated in this study.

Del(17p) is one of the typical aberration for B-CLL which is detected in 3%–8% naïve to treatment patients, and as frequent as up to 30% in patients with advanced, relapsed disease (11). Therefore, in order to distinguish the homozygotes from hemizygotes, we ran the copy number analysis applying TaqMan Copy Assays (Life Technologies, Carlsbad, CA, USA) of *APRIL* and *TACI* genes (located on chromosome 17p13.1 and 17p11.2, respectively) in the group of B-CLL patients. Single copy of *APRIL* and *TACI* genes was determined in 4.45% and 1.98% of the B-CLL patients, respectively. These samples were excluded from all analyses conducted in this study.

Chi-squared test, χ^2_{df}, was used to test the null hypothesis that cases and controls have the same distribution of genotype counts. In the case of small numbers, distribution of the test statistics was estimated numerically. Odds ratio (OR) was computed as the measure of effect size. Age at diagnosis was described by mean, standard deviation (SD) and range (minimum–maximum). Analysis of variance was used to test relations between selected polymorphisms and age at diagnosis. Homogeneity of variance among groups was verified with Fligner and Killeen method. Departure from Hardy–Weinberg equilibrium (HWE) was tested with chi-squared test and measured as $f = \frac{p_{ii} - p_i^2}{p_i(1-p_i)}$, where p_i and p_{ii} are allele i and genotype ii frequencies while $f < 0$ and $f > 0$ corresponds to deficiency and excess of homozygotes, respectively, and $f = 0$ in case of HWE. Haplotype frequencies (HFs) among SNPs were estimated with maximum-likelihood function (12). Measure for the estimation of pair-wise linkage disequilibrium (LD) was squared correlation between two SNPs, R^2 (13). Likelihood-ratio statistic, approximately chi-squared test, was used to test for differences in HFs between cases and controls, $LRS = 2(LL_{Cases} + LL_{Controls} - LL_{Combined})$. There were 20 hypotheses stating that H_i: distribution of genotypes in case and control groups is not different. The expected significance level for a single test was $0.05/20 = 0.0025$. Because of $LD \neq 0$ corrected $\alpha \approx 0.003$. This significance level was obtained based on 3000 steps (Monte Carlo simulation), where all $H_{i=1\ldots20}$ were true and tested in every step. Finally, if V is the number of hypotheses incorrectly rejected, then we find such α that $P(V < 1) \geq 1 - \alpha$. In case of modeling of the age at diagnosis according to *BAFF* rs9514828 and *BCMA* rs11570136 polymorphisms, genotypes were treated as ordinal variables according to the number of allele A (BCMA) and the number of allele T (BAFF). The quadratic contrasts in the model and interaction between them were tested with adjustment for gender.

It has to be mentioned that from the formal point of view none of the 20 SNPs examined in this study was associated with the risk of B-CLL. The significance level for this study was estimated numerically at $\alpha = 0.003$. However, the high value of the global chi-squared statistic ($\chi^2_{df=38} = 52.65$; $P = 0.0586$), obtained values of OR, our knowledge of the potential role of selected SNPs together with the results published by other groups indicate that some of the variants described below may be associated with the risk of B-CLL.

The distribution of genotypes of *BAFF* is presented in Table S1. Of the 4 *BAFF* SNPs tested, association with B-CLL was observed only for rs9514828 ($\chi^2_{df=1} = 3.946$; $P = 0.047$). The risk of developing B-CLL was 1.5 times lower for heterozygotes in this polymorphic site (OR = 0.68) in comparison with the CC homozygotes. It is also worth noticing that rs9514828CT genotype was more frequent among the controls than patients (54.7% *vs* 45.5%) and that the genotype distribution of this variant in controls was characterized by overrepresentation of rs9514828CT genotype if compared with the frequency estimated by the HWE [$f = -0.11$; confidence interval (CI) 95% = −0.22; 0.004]. Although the obtained *P-value* ($P = 0.047$) did not reach the statistical significance for this study, the results presented by other groups did not allow us to completely exclude the possibility of the association between this variant and the risk of B-CLL.

The distribution of genotypes of *APRIL* is presented in Table S2. Interesting results were obtained for rs3803800. The genotypes distribution of this variant differed markedly between the patients and the controls ($\chi^2_{df=2} = 9.669$; $P = 0.008$). We assumed that the rs3803800 might be associated with B-CLL risk. Indeed, homozygotes AA were more frequent in the patients than in the controls (8.0% *vs* 3.4%) and possessed significantly higher risk of B-CLL than the reference group (homozygotes GG) (OR = 2.09; CI 95% = 0.92; 4.74). Next, we divided the subjects into two groups, the carriers of G allele (GG or GA) and homozygotes AA, to estimate the risk of B-CLL for these groups. The analysis indicated that the subjects with rs3803800AA genotype had much higher risk of B-CLL (OR = 2.45; CI 95% = 1.10; 5.49; $P = 0.025$).

Table 1 Characteristic of B-CLL patients

Variables		Mean	SD	Minimum	Maximum		
Age at diagnosis*	Female ($n=78$)	64.6	10.5	42	86		
	Male ($n=99$)	61.6	10.8	36	83		
	All ($n=177$)	62.9	10.7	36	86		
Rai stage*	Score	0	I	II	III	IV	Σ
	N	55	42	24	13	40	174
	Per cent	31.6	24.1	13.8	7.5	23	100
	Cumulative (%)	31.6	55.7	69.5	77	100	—

SD, standard deviation.
*Not for all patients data were available.

The distribution of five examined SNPs of *TACI* is presented in Table S3. None of the examined SNPs was significantly associated with the B-CLL risk. However, the considerable difference in the genotypes distribution between the B-CLL and the control groups for rs4985726 ($\chi^2_{df=2}=6.961$; $P=0.029$) was observed. These data suggest that rs4985726 may be considered as the potential B-CLL risk factor. The distribution of genotypes of *BAFF-R* and *BCMA* are presented in Tables S4 and S5, respectively. We did not find any of these SNPs to be associated with B-CLL.

The haplotypes distribution, estimated separately for all investigated genes, did not differ between the patient and control groups for any of examined SNPs. Because *APRIL* and *TACI* genes are both located on chromosome 17, we additionally carried out an analysis to estimate the difference between the B-CLL and the control group in the distribution of haplotypes of all investigated SNPs of *APRIL* and *TACI.* The distribution of haplotypes in both groups was similar ($\chi^2_{df=19}=22.23$; $P=0.273$). The analysis of LD for particular genes in our sample of Polish population showed: the presence of modest LD between: rs9514827 and rs9514828 ($R^2=0.39$) (*BAFF*); rs6002551 and rs7290134 ($R^2=0.32$); rs5996088 and rs7290134 ($R^2=0.35$) (*BAFF-R*) and between rs11552708 and rs6608 ($R^2=0.44$) (*APRIL*). The high value of LD was observed between two SNPs, rs8072293 and rs11656106 ($R^2=0.74$) (*TACI*).

Because BAFF, APRIL and their receptors exert their role by interactions with each other, we examined the combined influence of SNPs investigated in this study on the B-CLL susceptibility in the following combinations: 1) *BAFF* and *BAFF-R*; 2) *BAFF* and *TACI*; 3) *BAFF* and *BCMA*; 4) *APRIL* and *TACI*; 5) *APRIL* and *BCMA*. The analysis did not reveal any interaction among polymorphic variants examined here and B-CLL.

Next, *BAFF*, *APRIL*, *BAFF-R*, *TACI* and *BCMA* polymorphisms were subjected to the analysis for correlation with the clinical features such as: gender, age at diagnosis and the Rai stage. The association between two SNPs: rs9514828 (*BAFF*) and rs11570136 (*BCMA*) and the age at diagnosis was found ($F_{df=9;183}=2.643$; $P=0.0067$). This association was adjusted to the sex. Because of the interaction between these two SNPs ($P=0.0384$), their effects are not additive. Along with the increase in the number of allele A in rs11570136 we observed the increase in the age at diagnosis, however, this effect was noticeable only in rs9514828CT heterozygotes (Table S6). The rs9514828CT B-CLL patients with one A allele in rs11570136 (rs11570136TA) were diagnosed at 66.81 years, and patients with two alleles A (rs11570136AA) were diagnosed at 75.2 years compared with the group of patients with rs11570136TT genotype (63.9 years) (Figure 1) (Table S6). Thus, the average age at diagnosis for B-CLL patients with the complex genotype rs9514828CT/rs11570136AA was 75.2 years, and it was 12 years later than the average age at diagnosis for the B-CLL patients from this study (63.37 years). Even though one person can have 0, 1 or two alleles, it is possible to consider relationship between average number of alleles per person and the expected mean of age at diagnosis in the group of patients (Figure S1), (Table S6). None of the SNPs investigated in this study was associated with the Rai stage.

B-CLL is recognized as the most frequent leukemia of adult citizens of Western countries (14). Evidence from numerous studies demonstrate the strong inherited genetic component in the susceptibility to B-CLL (15, 16). The failure to identify a major disease-causing locus caused that the co-inherence of multiple low-risk variants is widely assumed model of disease susceptibility (17). Association study is the most common method for identifying prevalent low-risk variant by comparing the frequency of polymorphic genotypes in controls and patients (17).

The substantial body of research suggests that BAFF in combination with other factors may play a potent role in promoting development of B-cell lymphoproliferation (2, 18). With respect to that, Novak et al. (5) reported the elevated levels of sBAFF in patients with familial B-CLL in relation to healthy controls and individuals with sporadic B-CLL. Moreover, they showed the possible correlation between the elevated level of sBAFF with the presence of T allele at −871 (rs9524828) in the *BAFF* promoter (5). The reporter gene assays demonstrated the elevated promoter activity and *BAFF* transcription for the −871T allele in HL60, HEK-293, Jurkat E6-1 and HuT 102 cell lines (5, 19). The contradictory results were obtained by de Almeida and Petzl-Erler who showed that the average level

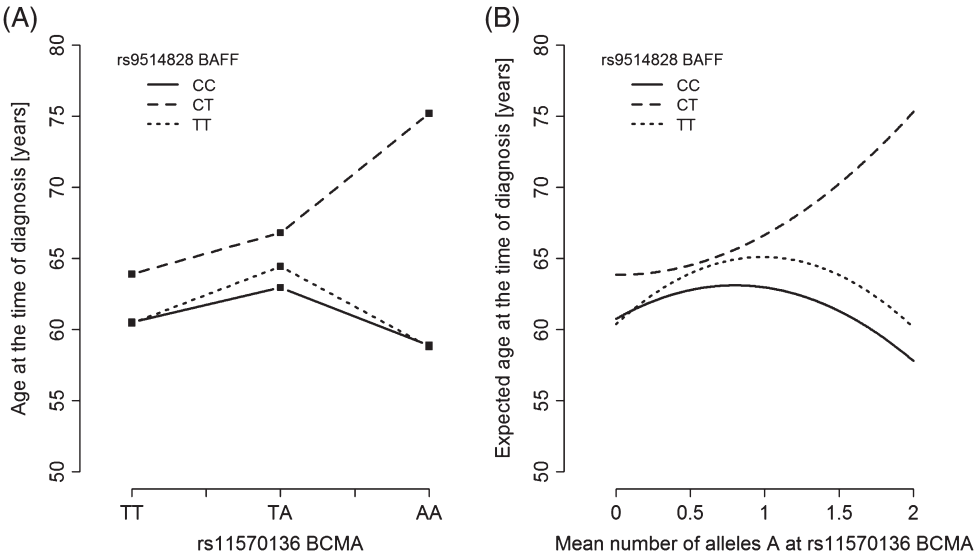

Figure 1 Means of the age at diagnosis according to rs9514828 of *BAFF* and rs11570136 of *BCMA* polymorphisms. (**A**) Observed and (**B**) expected means based on fitted model depending on the mean number of allele A per person at rs11570136 of *BCMA* and genotype at rs9514828 of *BAFF*. For example, the age at diagnosis for B-CLL patients with the genotype rs9514828CT/ rs11570136AA was 75.2 compared with 60.5 for the patients with the genotype rs9514828CC/ rs11570136TT.

of BAFF protein on CD8 + T cells, monocytes and NK cells isolated from healthy, untreated donors was significantly higher for the CC genotype at −871 position of *BAFF* promoter in comparison with T+ genotypes (20). In addition, the increased messenger RNA level was presented in peripheral blood mononuclear cells (PBMC) of the subjects with the CC genotype (20). What is more, the different impact on disease risk was observed for −871 C>T polymorphism. The T variant have been reported as the risk variant of the familial B-CLL (5) and high risk allele of pSS (3) and contradictory, as a favorable allele for T-cell lymphoma (TCL) patients survival (19) and as associated with lower susceptibility to pemphigus foliaceus (21). Here, we observed differences in the genotype distribution of −871C>T variant between the B-CLL and control groups ($P = 0.047$), arose because of the underrepresentation of −871CT heterozygotes in the B-CLL group. Based on our observations one may assume that the heterozygosity at −871 in *BAFF* promoter may protect from incidence of B-CLL (OR = 0.68). This observation is interesting in the light of the presented above contradictory results regarding protective or promoting role of T and C variants in different diseases and their opposite effect on *BAFF* promoter activity.

According to our best knowledge, there are only two other, earlier mentioned, reports of the same group (5, 7) which investigated the association of −871 C>T variant with the risk of B-CLL. First, they showed the possible association of −871T allele with familial B-CLL (5); however, they did not confirm this association with NHLs nor with B-CLL subtype in a later study (7). Therefore, there is the need to conduct additional studies on larger populations to determine the real impact of −871 C>T SNP on B-CLL susceptibility, progression and the level of sBAFF in B-CLL patients.

Recent publications emphasize the importance of genetic variations in genes encoding not only BAFF but also its receptors (BAFF-R, TACI and BCMA) in the pathogenesis of NHLs (2). Hilderbrant et al. (22) described a novel variant (rs61756766) in *BAFF-R* gene which was presented in both germline and tumor tissues from a subset of NHLs (DLBC; FL; lymphoplasmacytic lymphoma and MALT) patients (2, 22). This variant introduces an amino acid His159Tyr (H159Y) substitution in the cytoplasmic tail of BAFF-R and increased recruitment of TRAF3, TRAF2 and TRAF6 (2, 22). The average frequency of this variant in NHLs was 4.5% and even higher 10% in follicular lymphoma. This group did not find this genetic variant in any of 100 normal tissues screened (22), although the NCBI SNP database reports the 1.5% and 1.9% frequency of this variant. In our study, the H159Y was present in 5.3% of B-CLL patients and 3% of control subjects. Similarly to Hilderbrant et al. (22), we observed the absence of rs61756766 TT homozygotes in both examined groups. Our analysis showed that heterozygotes CT may have 1.8 higher risk to develop B-CLL than CC homozygotes (OR = 1.79; CI 95% = 0.73; 4.39). Unfortunately, because of a very low frequency of T allele ($\hat{\pi} = 0.015$) in population, we cannot confirm this association without the risk of error higher than expected $\alpha = 0.05$. The power analysis indicated that at least 1200 subjects in each group needs to be tested to achieve a 0.8 (80%) power to detect OR = 1.80. We also examined rs6002551 of *BAFF-R* which was previously found (*P-trend* = 0.0074) to be associated with NHLs risk (6). In our study, rs6002551 was not

associated with B-CLL which may be caused by the fact that this is not a risk variant for this subtype of NHLs.

According to our knowledge, this study is the first to investigate the association between *APRIL* gene polymorphisms and B-CLL risk. The distribution of rs3803800 genotypes differed markedly between the patients and controls ($P = 0.008$). Interestingly, in the patient group we observed lower frequency of heterozygotes rs3803800GA (28.35% *vs* 39.5%) and at the same time the higher frequency of homozygotes rs3803800AA (8.0% *vs* 3.4%) in comparison with the control group. The subjects with AA genotype had much higher risk of B-CLL (OR = 2.45; CI 95% = 1.10; 5.49; $P = 0.025$). Previously, this variant was reported in the preliminary study to be associated with biological response to atacicept in B-CLL patients (23). Rs3803800 had been also shown to be associated with the risk of SLE and higher serum level of APRIL (24, 25). Moreover, genom-wide association (GWA) study on levels of serum total protein, albumin and non-albumin protein in Japanese reported rs3803800 to be associated with serum level of IgG, IgM and IgA (25). The rs3803800 G>A is a missense variant (Asn96Ser). This variant is located near to the APRIL furin cleavage site (104Arg/105Ala) and it is predicted by *in silico* analysis to alter splicing of *APRIL* (9, 25). Furthermore, this GWAS reported also association of rs4985726 in *TACI* gene with serum level of IgG and IgM (25).

In this study, we noticed the considerable difference in the genotypes distribution between the B-CLL and the control groups for rs4985726 ($P = 0.029$). What is more, this result corresponded with a clear deviation from the HWE in the B-CLL group ($f = 0.2$; $P = 0.017$). At the same time, the frequency of genotypes in the control group was close to that expected from HWE estimation ($f = -0.018$; $P = 0.825$). This result may imply an association between rs4985726 and B-CLL since according to Salanti et al. (26) in the presence of an association, cases do not need to be in HWE, and in fact screening with HWE of data sets of affected individuals has been proposed as a relatively efficient method for detecting gene–disease association (26).

The BCMA has been reported to be expressed on the number of hematologic malignancies such as Hodgkin's lymphoma (HL) and NHLs (27). The genetic variants rs2017662 and rs2071326 in the *BCMA* gene were shown to be associated with the survival of patients with TCL (28). Here, none of the examined SNPs was found to be associated with the risk of B-CLL. However, we found an interesting interaction between rs9514828 of *BAFF* and rs11570136 of *BCMA* associated with the age at diagnosis of B-CLL. We observed that the average age at diagnosis for B-CLL patients with the complex genotype rs9514828CT/rs11570136AA was 75.2 years, and it was 12 years higher than the average age at diagnosis for the all B-CLL patients from this study (63.37). This difference in the age at diagnosis was estimated with low precision because of the small number of patients possessing the combination of rare genotypes and has to be further investigated in independent studies.

To sum up, similarly to other reports, we found that rs9524828 (*BAFF*) may be associated with the risk of B-CLL. In addition, our research provides some evidence for possible association between the two more variants, rs3803800 (*APRIL*) and rs4985726 (*TACI*), and susceptibility to B-CLL. We also noticed an interesting interaction between two SNPs, rs9514828 (*BAFF*) and rs11570136 (*BCMA*), associated with the age at diagnosis of B-CLL.

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Conflict of interest

The authors have declared no conflicting interests.

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Supporting Information

The following supporting information is available for this article:

Fig. S1. Expected means of the age at diagnosis in group of patients according to the mean number of allele C of rs9514828 of B-cell activating factor (*BAFF*) and the mean number of allele A of rs11570136 of B-cell maturation antigen (*BCMA*) per person in the group.

Table S1. Genotype distribution of the B-cell activating factor (*BAFF*) (*TNFSF13B*; 13q33.3) polymorphisms in patients and controls

Table S2. Genotype distribution of the a proliferation-inducing ligand (*APRIL*) *(TNFSF13*; 17p13.1) polymorphisms in patients and controls

Table S3. Genotype distribution of the transmembrane activator and calcium modulator and cyclophilin-ligand interactor (*TACI*) *(TNFRSF13B*; 17p11.2*)* polymorphisms in patients and controls

Table S4. Genotype distribution of the BAFF receptor (*BAFF-R*) (*TNFRSF13C*; 22q13.2) polymorphism in patients and controls

Table S5. Genotype distribution of the B-cell maturation antigen (*BCMA*) (*TNFRSF17*; 16p13.13) polymorphisms in patients and controls

Table S6. Conditional and marginal means, standard deviations (SD) and ranges (minimum–maximum) of age at diagnosis according to B-cell activating factor (*BAFF*) rs9514828 and B-cell maturation antigen (*BCMA*) rs11570136 polymorphisms