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Polymorphisms and haplotypes of the *NBS1* gene in childhood acute leukaemia

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ARTICLE INFO

Article history:
Received 4 April 2008
Received in revised form 4 June 2008
Accepted 20 June 2008
Available online 6 August 2008

Keywords:
Polymorphism
NBS1 gene
Haplotype
Childhood leukaemia

ABSTRACT

DNA repair gene polymorphisms and mutations may influence cancer risk. The product of the *NBS1* gene, nibrin, is functionally involved in the double-strand DNA break repair system. Heterozygous, germline mutations of the *NBS1* gene are associated with an increased risk of tumours. Thus, common polymorphism and haplotypes of *NBS1* may contribute to the risk of cancer. This study verified whether polymorphisms of the *NBS1* gene may influence susceptibility to the development of childhood acute leukaemia. We genotyped six polymorphisms of the *NBS1* gene in 157 children with acute leukaemia and 275 controls. The TT genotype of c.2071-30A > T polymorphism was higher in leukaemia patients than in controls. Genotyping data from the six polymorphic loci in *NBS1* in leukaemia patients and controls were used to impute haplotypes. Two of the evaluated haplotypes were associated with significantly increased leukaemia risk ($P = 0.0038$ and $P < 0.0001$). Our results suggest that some specific haplotypes of the *NBS1* gene may be associated with childhood leukaemia.

1. Introduction

Damage of DNA double-strand break repair may lead to genomic instability, thus increasing the risk of cancer development. The product of the *NBS1* gene, nibrin (p95, *NBS1*), is the key regulator of the MRE11/RAD50/nibrin (M/R/N) protein complex involved in the repair of DNA double-strand breaks (DSBs), telomere maintenance, immunoglobulin class switching, meiotic recombination and DNA damage response.[1,2] Nibrin directs the M/R/N complex to the sites of DNA damage and promotes the DNA binding and nuclease activity of the complex. Nibrin plays a role not only in active response to DSBs but also in the activation of cellular signalling cascades. Recent studies have indicated that nibrin is required not only for the activation of ATM (ataxia telangiectasia mutated) kinase in the presence of mutagenic DSBs, but also for the efficient downstream ATM phosphorylation of many targets critical for the cell cycle.[3–6]

The *NBS1* gene is located on chromosome band 8q21.3[1,7,8] and contains 16 exons. Biallelic mutations in *NBS1* cause Nijmegen Breakage Syndrome (NBS). NBS is an inherited chromosomal instability disorder which is characterised by immunodeficiency and increased predisposition to malignancy. Most patients are of Eastern and Central European origin, especially Polish. The most frequent homozygous mutation of the *NBS1* gene, called Slavic and observed in 90% of patients, is 657del5, i.e. deletion of ACAAA.[9]

About 40% of NBS patients develop malignancy before the age of 21. Approximately 70% of NBS patients who develop cancer have non-Hodgkin lymphomas (NHLs). The high

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.06.026

incidence of malignancies in close relatives of NBS patients[10] suggests that a heterozygous mutation of the *NBS1* gene may increase cancer risk. Heterozygous carriers of the *NBS1* 657del5 mutation have an increased risk of malignant tumour development, especially of breast cancer[11,12], prostate[13], and colon and rectum cancer.[11] The association of the 657del5 mutation with increased risk of haematological cancer has been shown recently.[11,14] In lymphoma and non-Hodgkin lymphoma, several studies did not reveal any association between the *NBS1* gene mutations and cancer.[15–18] We published three studies in which we found that the germline I171V mutation in the *NBS1* gene may be considered as a risk factor in the development of acute lymphoblastic leukaemia (ALL) and solid malignant tumours including larynx, breast and colorectal cancer.[19–21] On the basis of these studies, it is likely that common polymorphism and haplotypes of *NBS1* contribute to the risk of cancer. Only a few studies have been conducted so far to investigate the association of polymorphisms and haplotypes in the *NBS1* gene with cancer. Publications on *NBS1* polymorphisms have focused on the 553G > C polymorphism, in exon five of the *NBS1* gene, and breast, bladder and lung cancer risk assessments.[22–26] Furthermore, most of them have evaluated only polymorphisms, not haplotypes of the *NBS1* gene, and results have been inconsistent. Reports on the association between DNA repair polymorphisms and childhood acute leukaemia are very scanty. That is why in our study we analysed six polymorphisms and haplotypes of the *NBS1* gene in Polish children with acute leukaemia. Although several studies have focused on associations between the *NBS1* gene and cancer risk, especially haematologically, its role as a cancer risk gene is still unclear.

2. Materials and methods

2.1. Materials

Blood samples were obtained from 157 children aged 1–18 years who were diagnosed with lymphoid malignancies and were treated at the Oncology and Haematology Clinic in Poznań. 75% of the venous blood samples were obtained from patients in remission. The diagnosis of leukaemia was made by using the French-American-British (FAB) criteria, after conventional cytochemical and surface-marker analysis. Among the 157 children, 145 were diagnosed with ALL. The ALL group was pooled from patients from our previous study[19] and the present work. Using the FAB classification, 107 of the patients had L1 lymphoblast-morphology, 20 had L1/L2 morphology, 12 had L2 morphology and five were of L3 morphology. Approximately 94% of the cases were of the B-cell precursor type, and of these, 121 patients expressed the CD10 antigen, 10 had a pre-B immunophenotype, five patients had B-cell leukaemia and six patients had T-cell leukaemia. Twelve children were diagnosed with acute myelogenous leukaemia (AML). Using the FAB criterion, seven of the patients with AML were of M1 morphology and five had M0, M2, M2/M3, M3 and M5 morphology, respectively.

The research protocol was approved by the Ethics Committee, University of Medical Sciences, Poznań. 275 anonymous blood samples, collected on Guthrie cards drawn from the newborn screening programme of the Wielkopolska province, were used as controls.

2.2. Methods

Genomic DNA was extracted from whole blood by using a DNA extraction kit (Genomic Mini, A&A Biotechnology). DNA from Guthrie cards was isolated by boiling a piece of Guthrie card at 95 °C for 90 min. DNA was genotyped for six polymorphisms in the *NBS1* gene: c.102G > A, c.553G > C, c.1124 + 18C > T, c.1197T > C, c.2016A > G, and c.2071-30A/T (Table 1). The *NBS1* gene polymorphisms selected for our study had minor allele frequencies (>0.4) in order to achieve sufficient statistical power. Genotyping was performed by the PCR-SSCP method. PCR was used to amplify sequences containing polymorphisms investigated in our study. A set of six specific primer pairs flanking each of these exons were used as described previously.[19] The reaction was carried out in a total volume of 25 μl, containing 100 ng DNA, *Taq* DNA Polymerase, buffer with 15 mM $MgCl_2$ (Eppendorf, Hamburg, Germany), dNTPs (Sigma-Aldrich, Steinheim, Germany), and primers (Oligo, Warsaw, Poland). PCR product and loading buffer were mixed at a ratio of 1:3 and denatured for 5 min at 95 °C, cooled, and separated on 7% nondenaturing polyacrylamide gel (w/v) with absence or presence of 5% glycerol (v/v) at 4 °C or room temperature. Control samples with known polymorphisms were run in parallel. Silver staining was used for detection of single-strand DNA in polyacrylamide gels.

The statistical differences in polymorphisms between the studied group and controls were statistically evaluated by the chi-square test,. The differences were considered significant if the value of probability (*P*) did not exceed 0.05. In case of polymorphisms, the wild-type genotype/allele served as a reference category. Genotype frequencies observed and expected from the Hardy–Weinberg equilibrium were compared with the standard chi-square test. Logistic regression analysis was used to calculate the odds ratios (ORs) and their 95% confidence intervals (CIs) in case-control comparisons. Associations

Table 1 – *NBS1* gene polymorphisms tested in controls and acute leukaemia patients

Polymorphisms	Exon/Intron	Consequence	Type	Location in the protein
c.102G > A	2	p.L34L	synonymous	FHA domain
c.553G > C	5	p.E185Q	non-synonymous	BRCT domain
c.1124 + 18C > T	IVS9	–	–	–
c.1197T > C	10	p.D399D	synonymous	central region
c.2016A > G	13	p.P672P	synonymous	MRE11 binding domain
c.2071-30A > T	IVS13	–	–	–

among investigated polymorphisms were investigated by studying linkage disequilibrium (LD) measures. On the basis of the genotyping data of subjects included in the study, we calculated the Levontin's D' values and correlation coefficient r^2 between any pair of these six polymorphisms. These associations were also tested for significance by means of the chi-square test. The D' and r^2 were used to indicate the strength of LD. The haplotype frequencies were calculated based on the maximum likelihood method. Finally, the associations between haplotypes and the disease were checked. Specific haplotype frequencies were compared among patients and controls (chi-square test). Statistical analyses were done by means of the SAS 9.1 system-module GENETICS; procedures: ALLELE, HAPLOTYPE and CASECONTROL.

3. Results

Polymorphism and haplotype analysis of the *NBS1* gene was performed on DNA isolated from 157 children with acute leukaemia and from 275 control samples. One new polymorphism: c.2071-30A > T and five described previously (c.102G > A, c.553G > C, c.1124 + 18C > T, c.1197T > C, c.2016A > G) were detected in both groups. The percentage of leukaemia cells in the samples obtained from children with

Table 2 – The allele frequency distribution and results of logistic regression analysis (odds ratio [OR] and 95% confidence interval [CI]) of the studied *NBS1* gene polymorphisms in controls and acute leukaemia patients

Polymorphism	Allele	Leukaemia *n* [%]	Controls *n* [%]	OR [95% CI]	*P*
c.102G > A	G	195 [62]	361 [66]	1[a]	0.2968
	A	119 [38]	189 [34]	1.166 [0.8739–1.555]	
c.553G > C	G	195 [62]	356 [65]	1[a]	0.44
	C	119 [38]	194 [35]	1.12 [0.8401–1.493]	
c.1124 + 18C > T	C	198 [63]	341 [62]	1[a]	0.7576
	T	116 [37]	209 [38]	0.9559 [0.7176–1.273]	
c.1197T > C	T	175 [56]	321 [58]	1[a]	0.4519
	C	139 [44]	229 [42]	1.113 [0.8415–1.473]	
c.2016A > G	A	196 [62]	358 [65]	1[a]	0.4312
	G	118 [38]	192 [35]	1.123 [0.8417–1.497]	
c.2071-30A > T	A	192 [61]	363 [66]	1[a]	0.1522
	T	122 [39]	187 [34]	1.233 [0.9253–1.644]	

a Reference category.

Table 3 – The genotype frequency distribution and results of logistic regression analysis (odds ratio [OR] and 95% confidence interval [CI]) of the studied *NBS1* gene polymorphisms in controls and acute leukaemia patients

Polymorphism	Genotype	Leukaemia *n* [%]	Controls *n* [%]	OR [95% CI]	*P*
c.102G > A	GG	64 [41]	116 [42]	1[a]	
	GA	67 [43]	129 [47]	0.9414 [0.6156–1.439]	0.7803
	AA	26 [16]	30 [11]	1.571 [0.8556–2.884]	0.1435
	GA + AA	93 [59]	159 [58]	0.9433 [0.6333–1.405]	0.7738
c.553G > C	GG	66 [42]	111 [40]	1[a]	
	GC	63 [40]	134 [49]	0.7907 [0.5158–1.212]	0.2809
	CC	28 [18]	30 [11]	1.57[0.8627–2.856]	0.1382
	GC + CC	91 [58]	164 [60]	0.9332 [0.6267–1.390]	0.7335
c.1124 + 18C > T	CC	69 [44]	105 [38]	1[a]	
	CT	60 [38]	131 [48]	0.697 [0.4530–1.072]	0.0999
	TT	28 [18]	39 [14]	1.093 [0.6161–1.937]	0.762
	CT + TT	88 [56]	170 [62]	0.7877 [0.5290–1.173]	0.2398
c.1197T > C	TT	49 [31]	96 [35]	1[a]	
	TC	77 [49]	129 [47]	1.169 [0.7493–1.825]	0.4905
	CC	31 [20]	50 [18]	1.215 [0.6903–2.137]	0.4996
	TC + CC	108 [69]	179 [65]	1.182 [0.7775–1.797]	0.4336
c.2016A > G	AA	66 [42]	115 [42]	1[a]	
	AG	64 [41]	128 [46]	0.8712 [0.5688–1.334]	0.526
	GG	27 [17]	32 [12]	1.47 [0.8108–2.666]	0.203
	AG + GG	91 [58]	160 [58]	1.182 [0.7775–1.797]	0.4336
c.2071-30A > T	AA	64 [41]	117 [43]	1[a]	
	AT	64 [41]	129 [47]	0.907 [0.5915–1.391]	0.6543
	TT	29 [18]	29 [10]	**1.828 [1.005–3.325]** *	**0.0466**
	AT + TT	93 [59]	158 [57]	1.076 [0.7226–1.602]	0.7182

a Reference category.
* Statistically significant ($P < 0.05$).

acute leukaemia had no impact on the detection of polymorphisms. The distributions of genotype and allele frequencies for each of the analysed polymorphisms among studied groups are shown in Tables 2 and 3, respectively. The TT genotype of c.2071-30A > T polymorphism was increased in leukaemia patients, as compared to controls ($P = 0.04$, OR = 1.828 (95%CI: 1.005–3.325)) (Table 2). No significant differences in allele and genotypes frequencies at the other five polymorphic sites were observed between leukaemia patients and controls. The observed genotype frequencies of c.102G > A, c.553G > C, c.1124 + 18C > T, c.1197T > C, c.2016A > G, and c.2071-30A > T polymorphisms were all in agreement with the Hardy–Weinberg equilibrium in the control subjects ($P = 0.50839$, 0.26564, 0.8558, 0.56374, 0.73232 and 0.45341, respectively).

On the basis of the genotyping data of subjects included in this study, we calculated the D' values and correlation coefficient r^2 between any pair of these six polymorphisms. We observed differences in the level of linkage between the patients and controls. The LD analysis in leukaemia patients (Table 4) showed that locus c.102G > A was in LD with locus c.553G > C ($D' = 0.8568$, $r^2 = 0.7905$, $P < 0.0001$), with locus c.1124 + 18C > T ($D' = 0.8891$, $r^2 = 0{,}7523$, $P < 0.0001$), with locus c.1197T > C ($D' = 0.8853$ $r^2 = 0.3457$, $P < 0.0001$), and with locus c.2071-30A > T ($D' = 0.9305$, $r^2 = 0.7049$, $P < 0.0001$). Locus c.102G > A was in LD with locus c.1124 + 18C > T ($D' = 0.9$, $r^2 = 0.7777$, $P < 0.0001$), locus c.2016A > G ($D' = 0.9725$, $r^2 = 0.9329$, $P < 0.0001$) and with locus c.2071-30A > T ($D' = 0.9297$, $r^2 = 0.8301$, $P < 0.0001$). Locus c.1124 + 18C > T was in LD with locus c.2016A > G ($D' = 0.9153$, $r^2 = 0.8152$, $P < 0.0001$) and locus c.2071-30A > T ($D' = 0.8674$, $r^2 = 0.6937$, $P < 0.0001$). Locus c.2016G > A was in LD with locus c.2071-30A > T ($D' = 0.9433$, $r^2 = 0.8429$, $P < 0.0001$). In the controls (Table 5), we found that only two pairs of analysed polymorphisms were in LD: locus c.553G > C was in LD with locus c.2071-30A > T ($D' = 0.8521$, $r^2 = 0.6864$, $P < 0.0001$) and locus c.2061A > G was in LD with locus c.2071-30A > T ($D' = 0.8077$, $r^2 = 0.6266$, $P < 0.0001$). The D' values for other variants were between 0.4 and 0.79, with $P < 0.0001$. For loci c.102G > A and c.1197T > C, linkage in controls was weak ($D' = 0.2268$, $r^2 = 0.\ 0377$, $P < 0.0013$).

Table 5 – The Levontin's D′ values and correlation coefficient r^2 between pairs of studied NBS1 gene polymorphisms in controls and results of the chi-square test

Polymorphism pairs		D'	r^2	P
c.102G > A	c.553G > C	0.6949	0.4638	<0.0001
c.102G > A	c.1124 + 18C > T	0.5833	0.2906	<0.0001
c.102G > A	c.1197T > C	0.2268	0.0377	0.0013
c.102G > A	c.2016A > G	0.6485	0.4104	<0.0001
c.102G > A	c.2071-30A > T	0.6794	0.4542	<0.0001
c.553G > C	c.1124 + 18C > T	0.7234	0.4652	<0.0001
c.553G > C	c.1197T > C	0.5602	0.2397	<0.0001
c.553G > C	c.2016A > G	0.749	0.5520	<0.0001
c.553G > C	c.2071-30A > T	0.8521	0.6864	<0.0001
c.1124 + 18C > T	c.1197T > C	0.4028	0.1394	<0.0001
c.1124 + 18C > T	c.2016A > G	0.5911	0.3055	<0.0001
c.1124 + 18C > T	c.2071-30A > T	0.6417	0.3460	<0.0001
c.1197T > C	c.2016A > G	0.3953	0.1173	<0.0001
c.1197T > C	c.2071-30A > T	0.4479	0.1448	<0.0001
c.2016A > G	c.2071-30A > T	0.8077	0.6266	<0.0001

Genotyping data from six polymorphic loci in *NBS1* in leukaemia patients and controls were used to generate the haplotypes. The construction of haplotypes based on these six polymorphisms revealed the presence of 48 out of 64 possible haplotypes in patients and controls. However, haplotypes with frequencies of less than 0.01 in both groups were excluded from further analysis and are likely to be a result of genotyping errors or rare recombination events. Finally, we analysed eleven haplotypes (Table 6) and three main haplotypes made up the majority of patients and controls (GGCTAA 41%, ACTCGT 20%, GGCCAA 11%). Two of them, GGCTAA and ACTCGT, were associated with significantly increased leukaemia risk ($P = 0.0038$ and $P < 0.0001$, respectively). The GGTTAA, GGTCAA and AGCTAA haplotypes were more frequent in the controls, and the differences were statistically significant ($P = 0.0017$, $P = 0.0414$, and $P = 0.0319$).

4. Discussion

We previously reported that the I171V mutation in exon 5 of the *NBS1* gene may increase the risk of acute lymphoblastic

Table 4 – The Levontin's D′ values and correlation coefficient r^2 between pairs of studied NBS1 gene polymorphisms in acute leukaemia patients and results of the chi-square test

Polymorphism pairs		D'	r^2	P
c.102G > A	c.553G > C	0.8568	0.7905	<0.0001
c.102G > A	c.1124 + 18C > T	0.8891	0.7523	<0.0001
c.102G > A	c.1197T > C	0.8853	0.3457	<0.0001
c.102G > A	c.2016A > G	0.6708	0.8541	<0.0001
c.102G > A	c.2071-30A > T	0.9305	0.7049	<0.0001
c.553G > C	c.1124 + 18C > T	0.9	0.7777	<0.0001
c.553G > C	c.1197T > C	0.7119	0.3894	<0.0001
c.553G > C	c.2016A > G	0.9725	0.9329	<0.0001
c.553G > C	c.2071-30A > T	0.9297	0.8301	<0.0001
c.1124 + 18C > T	c.1197T > C	0.6816	0.3427	<0.0001
c.1124 + 18C > T	c.2016A > G	0.9153	0.8152	<0.0001
c.1124 + 18C > T	c.2071-30A > T	0.8674	0.6937	<0.0001
c.1197T > C	c.2016A > G	0.7253	0.3987	<0.0001
c.1197T > C	c.2071-30A > T	0.704	0.3965	<0.0001
c.2016A > G	c.2071-30A > T	0.9433	0.8429	<0.0001

Table 6 – *NBS1* haplotype frequencies and results of logistic regression analysis for their associations with acute leukaemia risk

Haplotype	Leukaemia patients	Controls	Combined	Chi^2	P
GGTTAA	0.00396	0.03902	0.02568	9.8302	0.0017*
GGTCAA	0.00573	0.02393	0.01637	4.1606	0.0414*
AGCTAA	0.0097	0.03239	0.02308	4.6048	0.0319*
GGCTAA	0.46658	0.3662	0.406	8.3914	0.0038*
ACTCGT	0.27597	0.15112	0.19601	19.7723	0*
ACCTGT	0.0037	0.01936	0.01329	3.7502	0.0528
GCTCGT	0.01216	0.02313	0.01916	1.2819	0.2575
ACCCGT	0.01987	0.01066	0.01398	1.2308	0.2672
ACTTGT	0.04065	0.05561	0.05176	0.955	0.3285
ACTCGA	0.00528	0.01096	0.0097	0.7296	0.393
GGCCAA	0.0996	0.10935	0.10494	0.2089	0.6476

* Statistically significant ($P < 0.05$).

leukaemia, larynx and breast cancer. In the present study, we have estimated the leukaemia risk associated with polymorphisms and haplotype in *NBS1*. The allele and genotype frequency distribution and logistic regression analysis of the studied *NBS1* gene polymorphisms, in controls and acute leukaemia patients, have showed a marginally significant association of the homozygous c.2071-30TT genotype ($P = 0.04$, OR = 1.828 (95%CI: 1.005–3.325)) with leukaemia. However, when alleles were combined in the form of haplotypes, the risk of leukaemia appeared to increase as the number of risk alleles increased.

In exon 2 of the *NBS1* gene, we genotyped the synonymous c.102G > A polymorphism (L34). No significant differences, in either allele or genotype frequencies at the locus, were observed between leukaemia patients and controls, $P = 0.2968$, OR = 1.166 (95% CI: 0.8739–1.555) and $P = 0.7738$, OR = 0.9433 (95% CI: 0.6333–1.405), respectively. These results are in agreement with other reports. In 91 North American patients with non-Hodgkin lymphoma, no association of the 102G > A polymorphism with the disease was found.[18] Allele and genotype frequencies of that polymorphism were not significantly different in epithelial ovarian and breast cancer patients compared with a control group.[27,28] Only in a population-based study in China were individuals homozygous for the A variant found to have an increased risk of lung cancer.[29] That polymorphism occurs in the conserved FHA domain which is important for nibrin function.[30] No information is available on the effect of the 102G > A polymorphisms on DNA repair activity.

Several studies on *NBS1* polymorphisms have focused on the non-synonymous c.553G > C polymorphism (E185Q), in exon 5 of the *NBS1* gene. SNPs that alter the amino acid sequence of the gene are of most interest, but results have been inconsistent. So far, no significant differences in genotype distribution and allele frequencies between the control and epithelial ovarian[27] and breast cancer patients[22,28,31] have been reported. However, Lu and colleaques[23] detected that the homozygous 553CC genotype and heterozygous 553GC genotype may contribute to sporadic breast cancer in young non-Hispanic white women. The 553G > C polymorphism has been previously evaluated in relation to lung cancer. The heterozygous GC genotype carriers had an increased risk of lung cancer in China.[29] In the total Swedish population, no significant effect of the 553G > C polymorphism on lung cancer risk has been observed.[32] A higher frequency of *p53* mutations has been reported among heterozygous and homozygous carriers of the c.553G > C polymorphism in lung cancer in the USA.[25] This observation has not been confirmed in bladder tumours.[33] However, the variant allele for the c.553G > C *NBS1* polymorphism has shown a marginal association with the occurrence of urinary bladder cancer.[24] In our previous study, we showed that heterozygous carries of the 553GC genotype had an increased risk of larynx cancer.[20] The lack of association between leukaemia risk and the c.553G > C polymorphism in the present study is in agreement with North American results obtained from NHL patients.[18] There is no other evidence on any effect of the c.553G > C locus on the risk of lymphoid malignancies. The functional consequences of the E185Q *NBS1* polymorphism are unknown. However, its location within the BRCA1 terminus (BRCT) domain may be related to some effect on protein function. The BRCA1 C-terminal domain is widely conserved in eukaryotic nuclear proteins related to the cell cycle, gene regulation and DNA repair.[34] The BRCT domain is required for either binding to histone gamma-H2AX or relocalisation of the hMRE11/hRAD50 nuclease complex to the vicinity of DNA damage.[35]

Interpretation of results for the other studied polymorphic sites is limited by the rather low number of data. The synonymous polymorphisms c.1197T > C (D399D) in exon 10 and c.2016A > G (P672P) in exon 13 of the *NBS1* gene have been investigated in a multicentre study of ovarian cancer from the UK, Denmark and the USA[27], a large case-control study of breast cancer in Europe[28], and a North American study of non-Hodgkin lymphoma.[18] Cerosaletti also analysed the intronic c.1124 + 18C > T polymorphism.[18] All these studies have not found any association between the *NBS1* c.1197T > C, c.2016A > G and c.1124 + 18 C > T polymorphisms and the studied cancer. Our study revealed no association of these polymorphisms with childhood leukaemia risk.

The c.2071-30A > T polymorphism, identified in our study, includes intronic sequence variants between exons 13 and 14 of the *NBS1* gene. The TT genotype of c.2071-30A > T polymorphism was increased in leukaemia patients in comparison with controls ($P = 0.04$, OR = 1.828 (95%CI: 1.005–3.325)). The association is marginally significant, but unique.

Few studies have been conducted so far to study the association of haplotypes in the *NBS1* gene with cancer. Lu and

colleaques[23] showed that an elevated risk of breast cancer was associated with haplotypes consisting of three *NBS1* polymorphisms. In another study on breast cancer risk, there were no significant differences in the estimated haplotypes between patients and controls.[28] The haplotype containing the variant alleles from the *NBS1* c.102G > A and 553G > C polymorphisms have been associated with an increased risk of lung cancer, compared with the most common haplotype.[29] Only in our study was genotyping data from six polymorphism loci in *NBS1* in leukaemia patients and controls used to generate the haplotypes. The differences were statistically significant ($P < 0.05$) in five out of eleven analysed haplotypes. Two of them, GGCTAA and ACTCGT, were associated with a significantly increased leukemia risk ($P = 0.0038$ and $P < 0.0001$, respectively). The GGTTAA, GGTCAA and AGCTAA haplotypes were more frequent in the controls and the differences were significant ($P = 0.0017$, $P = 0.0414$ and $P = 0.0319$). Interpretation of these results is limited by the rather low number of studies of this type. There is no evidence for association of haplotypes and known mutations in the *NBS1* gene. In the previous study we identified germline *NBS1* gene mutations in peripheral blood cells from nine of 135 ALL patients.[19] In the present study we were able to confirm the presence of the haplotypes associated with a significantly increased leukaemia risk in four out of nine heterozygous carriers of the *NBS1* gene mutations. We identified the GGCTAA haplotype in three ALL patients with confirmed germline 657del5, V210F (exon six) and D95N (exon three) mutations in the *NBS1* gene. The ACTCGT haplotype was confirmed in ALL patients with heterozygous R215W mutation in exon six of the *NBS1* gene. Thus, additional studies are required to determine the association between the haplotypes and any of the known mutations in the *NBS1* gene. Our analysis of the haplotype provided additional information on these polymorphisms as markers of genetic susceptibility and a more efficient method for assessing the genetic susceptibility of a candidate gene than any of the polymorphisms.

In conclusion, our results indicate that haplotypes containing the variant alleles from six studied *NBS1* polymorphisms may be related to increased susceptibility to childhood acute leukaemia.

Conflict of interest statement

None declared.

Acknowledgements

This work was done at the Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland and was supported by Ministry of Sciences and Informatics grant no. PBZ-KBN-09.0/P05/2003.

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