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A comprehensive study of polymorphisms in the *ABCB1*, *ABCC2*, *ABCG2*, *NR1I2* genes and lymphoma risk

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Abstract

Owing to their role in controlling the efflux of toxic compounds, transporters are central players in the process of detoxification and elimination of xenobiotics, which in turn is related to cancer risk. Among these transporters, ATP-binding cassette B1/multidrug resistance 1 (ABCB1/MDR1), ABCC2/multidrug resistance protein 2 (MRP2), and ABCG2/breast cancer resistance protein (BCRP) affect susceptibility to many hematopoietic malignancies. The maintenance of regulated expression of these transporters is governed through the activation of intracellular “xenosensors” like the nuclear receptor 1I2/pregnane X receptor (NR1I2/PXR). SNPs in genes encoding these regulators have also been implicated in the risk of several cancers. Using a tagging approach, we tested the hypothesis that common polymorphisms in the transporter genes *ABCB1*, *ABCC2*,

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Conflict of interest
The authors declare no conflict of interest.

ABCG2, and the regulator gene *NR1I2* could be implicated in lymphoma risk. We selected 68 SNPs in the 4 genes, and we genotyped them in 1,481 lymphoma cases and 1,491 controls of the European cases-control study (EpiLymph) using the Illumina™ GoldenGate assay technology.Carriers of the SNP rs6857600 minor allele in *ABCG2*, was associated with a decrease in risk of B-cell lymphoma (B-NHL) overall ($p<0.001$). Furthermore, a decreased risk of chronic lymphocytic leukemia (CLL) was associated with the ABCG2 rs2231142 variant ($p=0.0004$), which could be replicated in an independent population. These results suggest a role for this gene in B-NHL susceptibility, especially for CLL.

Keywords

Lymphoma; multidrug resistance 1 (MDR1); multidrug resistance protein 2 (MRP2); breast cancer resistance protein (BCRP); pregnane X receptor (PXR)

Introduction

Lymphomas are a heterogeneous group of malignancies arising in the lymphoid tissue, [1, 2] whose known risk factors include acquired or congenital immune system deficiency [3], familial history of a first degree relative with a hematological malignancy [4], and viral infections including Epstein Barr and hepatitis C virus infection. [5] Additionally, an increasing number of publications reported on the association between genetic variants and lymphoma risk, indicating the relevance of genetic variability in lymphomagenesis. [6, 7]

There is considerable epidemiological evidence that the process of detoxification and elimination of xenobiotics has implications for cancer risk. [8–11] Membrane transporters are central players in this task, owing to their role of controlling the efflux of toxic compounds and reducing the local cellular burden of xenobiotics. Transporter proteins reduce the entrance of harmful substances ("phase 0 metabolism") and increase the excretion of their detoxification products ("phase III metabolism"). [12] Among several players in this detoxifying/elimination process three ABC (ATP binding cassette) transporters ATP-binding cassette B1/multidrug resistance 1 (ABCB1/MDR1), ATP-binding cassette C2/multidrug resistance protein 2 (ABCC2/MRP2) and ATP-binding cassette G2/breast cancer resistance protein (ABCG2/BCRP), have a key role in protecting the organism in various tissues, including peripheral blood. [13–17]

The maintenance of regulated expression of both drug transporters and metabolizing enzymes is governed through the activation of intracellular "xenosensors". These ligand-activated transcription factors sense the intracellular level of xenobiotics and, upon ligand binding, translocate into the nucleus and transcriptionally activate genes involved in xenobiotic metabolism and transport. One of the best characterized of these sensors is nuclear receptor 1I2 (NR1I2), also known as pregnane X receptor (PXR), whose target genes include ABCB1, ABCC2 and ABCG2. [18]

Some polymorphisms in the ABC transporter genes and their regulators have a demonstrated effect on gene expression and/or protein function. [19, 20] Several have also been implicated in the risk of various cancers and they are thought to have a major impact on the genetic susceptibility to hematopoietic malignancies. However, results have been inconsistent thus far. [21–26]

To verify the impact of polymorphisms in genes related to xenobiotic metabolism on lymphomagenesis, using a tagging approach, we studied common genetic variability of *ABCB1*, *ABCC2*, *ABCG2* and *NR1I2*. The selection of the genes was driven by the

abundance of reports on how polymorphisms in the selected transporters can modify the protein function in comparison to other genes in the same family that were left out. We selected 68 single nucleotide polymorphisms (SNPs) which tag all frequent polymorphisms in the four genes, and we typed them in 1,481 lymphoma cases and 1,491 controls in the context of the EpiLymph study.

Design and Methods

Study population

Detailed information about the EpiLymph study is given elsewhere. [27] Briefly, this is a multi-centric case-control study carried out in six European countries (Spain, France, Italy, Germany, Ireland and Czech Republic) between 1998 and 2004. Incident cases with a diagnosis of a lymphoid malignancy were included in the study and categorized according to the WHO classification. [2] In Germany and Italy the corresponding controls were randomly sampled from population registries as described elsewhere and matched by age (± 5 years), sex and area of residence. [27] In the other study centers corresponding controls were recruited from the same hospitals as the cases, but had to have a diagnosis other than cancer, infectious diseases and immunodeficiency disorders. The participation rate was 88% in cases, 81% and 57% in hospital and population controls, respectively. Informed consent was sought from the 2,348 cases and 2,462 controls. All participants were asked to provide a blood sample for DNA extraction and took part in a standardized face-to-face interview requesting information on sociodemographic characteristics, familial and medical history, smoking and alcohol habits, a complete occupational history and data on leisure and occupational sun exposure. In each country the study was approved by the responsible ethics committee.

In this study 1,481 cases for which DNA was available were included and consisted of Hodgkin's Lymphoma (HL, n=272), T-cell non-Hodgkin Lymphoma (T-NHL, n=95), and B-cell non-Hodgkin Lymphoma (B-NHL, n=1,114). Among the B-NHL different entities were present: diffuse large B-cell lymphoma (DLBCL, n=408), follicular lymphoma (FL, n=200), chronic lymphocytic leukemia (CLL, n=340) including small lymphocytic lymphoma (SLL), marginal zone B-cell lymphoma (MZL, n=112), and mantle cell lymphoma (MCL, n=54). The corresponding EpiLymph controls (n=1,491) were frequency-matched by age, sex and study center. Details regarding the main characteristics of the study population used for the statistical analysis and the distribution of cases are presented in Table 1.

Selection of tagging single nucleotide polymorphisms

We aimed at surveying the entire set of common genetic variants in the *ABCB1, ABCC2, ABCG2* and *NR1I2* genes. All polymorphisms in the candidate gene regions (including 5 kb upstream of the first known exon and 5 kb downstream of the last known exon of each gene), with minor allele frequency (MAF) ≥5% in Caucasians from the International HapMap Project (version 22; http://www.hapmap.org), were included. Tagging SNPs were selected with the use of the Tagger program within Haploview (http://www.broad.mit.edu/mpg/haploview/;http://www.broad.mit.edu/mpg/tagger/; [28, 29]) using pairwise SNP selection with a minimum r^2 threshold of 0.8. This resulted in a selection of 15 tagging SNPs for *ABCG2*, with a mean r^2 of the selected SNPs with the SNPs they tag of 0.963, 23 tagging SNPs for *ABCB1*, with a mean r^2 of 0.956, 12 tagging SNPs for *ABCC2*, with a mean r^2 of 0.981, 14 tagging SNPs for *PXR*, with a mean r^2 of 0.984. Therefore, the selected SNPs capture over 95% of the known common variability in these genes. Considering that the genomic regions of the three genes are characterized by high levels of linkage disequilibrium (LD), we postulate that such SNPs are also likely to tag

any hitherto unidentified common SNPs in the respective genes. Since rs2725256 showed a poor Illumina score (0.76) we added rs1564481, which is in almost complete LD with the previous one (r^2=1). Moreover rs2231142 was added, due to its demonstrated functional relevance. [30] Online Supplementary Table S1 shows details of the SNPs included in the study.

Sample preparation and genotyping

DNA was extracted from blood clots (Gentra Puregene Blood Kit, Qiagen, Hilden, Germany). The order of DNA samples of cases and controls was randomized on PCR plates in order to ensure that an equal number of cases and controls could be analyzed simultaneously. Genotyping was carried out using the Illumina™ GoldenGate technology (San Diego, CA, USA), according to the protocol specified by the manufacturer. For quality control 3% of the samples were replicated.

Data filtering and statistical analysis

Samples where more than 4% of the SNPs had failed were dropped from the analyses (65 cases and 44 controls), 2 additional cases were excluded from the analysis due to missing age information. We then filtered data to remove poorly performing SNPs: all SNPs that failed on at least 5% of the samples (n=5) were set to missing. Statistically significant ($p<10^{-5}$) deviation from Hardy-Weinberg equilibrium (HWE) among controls was tested as well, but it did not lead to elimination of any SNP. After data filtering for quality control, 63 SNPs were analyzed in 1414 lymphoma cases and 1447 controls.

The association between genetic variants and risk of HL, T-NHL and B-NHL, as well as with the most common B-NHL entities was estimated by calculating Odds Ratios (OR), the corresponding 95% confidence intervals (CI) and *p*-values using unconditional logistic regression. The dominant, co-dominant and log-additive model of inheritance were used, and the homozygous genotype of the common allele was set as reference. All calculations were adjusted for age (continuous), sex and study centre.

In order to take into account the large number of tests performed in this candidate gene approach, we calculated for each gene the number of effective independent variables, M_{eff}, by use of the SNP Spectral Decomposition approach (*simpleM* method). [31] We obtained a gene-wise M_{eff} value for each gene and also a study-wise M_{eff} value, by adding up the gene M_{eff}'s. We calculated M_{eff} values for each candidate gene separately and for the whole study (by adding the individual gene M_{eff} values). The study-wise M_{eff} was 51. We therefore used a study-wise significance p-value threshold of 0.05/51=0.001.

As a sensitivity analysis for B-NHL we performed stratified analysis by gender and age, while the cut point for age was set at 50 years, according to previous publications by Wang *et al.* [4] Among the HL cases, the cut point was set at the age of 40years, due to the typical bimodal age distribution. To evaluate heterogeneity across countries, we used the likelihood ratio test to compare the models with and without an interaction term between country and SNP.

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc. Cary, NC).

Meta-analysis

For replication of the strongest associations identified in the present study population (EpiLymph), information on CLL risk was obtained from a NHL GWAS (SF1; 211 CLL cases and 750 controls) [7] and information of a GWAS on CLL (Mayo-GEC; 407 CLL cases and 296 controls)[32]. To compare the three studies, allelic odds ratios were estimated.

Furthermore, a meta-analysis was performed using the "meta" and "rmeta" packages of the statistical software environment R (2.13.0) [33]. We used the random effects model for combining the study estimates. The individual study results and the combined odds ratios and 95% CIs were presented in forest plots.

Haplotype reconstruction

Haplotype blocks were constructed from the control genotyping data using SNPtool (http://www.dkfz.de/de/molgen_epidemiology/tools/SNPtool.html, [34]) and the algorithm implemented in Haploview based on confidence bounds by Gabriel *et al.* [35] The following cut-off values were used: MAF>5%, HWE $p \geq 0.01$ and 75% of non-missing genotypes. Maximum likelihood estimates of the haplotype frequencies were generated with an expectation-maximization based algorithm implemented in the *PROC HAPLOTYPE* procedure of SAS. Unconditional logistic regression adjusted for age (continuous), sex and study centre was used to calculate risk estimates. The most frequent haplotype was set as the reference, whereas haplotypes with a frequency below 0.05 were declared as rare haplotypes and combined.

Gene-gene interactions

The nonparametric Multifactor Dimensionality Reduction (MDR) approach was selected to complement logistic regression for the analysis of gene–gene interactions. The details of MDR are described elsewhere. [36] Briefly, MDR is a data reduction approach that seeks to identify combinations of multilocus genotypes and discrete environmental factors that are associated with either high risk or low risk of disease. MDR defines a single variable that incorporates information from several loci and/or environmental factors. This new variable can be evaluated for its ability to classify and predict outcome risk status using cross-validation and permutation testing. The MDR software is available from http://www.epistasis.org.

Results

Main effects of genotyped single nucleotide polymorphisms

Twelve SNPs showed statistically significant associations at the conventional *p*-value of 0.05, with B-NHL (overall) and HL considering the three different inheritance models (dominant, co-dominant and log-additive). Online Supplementary Table S2 shows the corresponding results for all analyzed SNPs in all histological subtypes.

One SNP, rs6857600, situated in the *ABCG2* gene, was study-wise significantly associated with a 24% risk reduction for B-NHL. Carriers of at least one A allele were associated with a decreased risk of B-NHL (OR=0.75, 95%CI 0.64–0.90, p=0.001). Furthermore several other associations approached study-wise significance as detailed in Table 2, where SNPs associated with B-NHL, HL or CLL risk (p<0.01) are presented.

We have also analyzed the SNPs in each histological subtypes of B-NHL. Two SNPs belonging to the *ABCG2* gene showed a study-wise association with disease risk. Heterozygous carriers of the SNP rs1481012 (OR_{AG}=0.50, 95%CI 0.33–0.74, p=0.0005; $OR_{AG/GG}$=0.54, 95%CI 0.37–0.78, p=0.001), and heterozygous carriers of the rs2231142 SNP (OR_{CA}=0.49, 95%CI 0.33–0.73, p=0.0004, $OR_{CA/AA}$ =0.53, 95%CI 0.36–0.77, p=0.001) were associated with a decreased CLL risk The two SNPs are in high linkage disequilibrium with each other (r^2=0.989).

For all the other subtypes no association was found at the study-wise significance threshold, but several were significant at the canonical *p*-value of 0.05, as shown in Online Supplementary Table S2.

We also stratified the analysis by age and gender, but we did not find any further statistical study-wise association in the different strata. Results are shown in Online Supplementary Table S4 and S5.

Results of the replication and meta-analysis

For the purpose of validation of SNPs significantly associated with B-NHL or CLL risk, allelic odds ratios were calculated with genotyping information of two GWAS on NHL and CLL [7, 32]. In the Mayo-GEC study two *ABCG2* SNPs showed a statistically significant association with decreased risk of CLL, with ORs (rs1481012: OR=0.69, 95% CI 0.49–0.97, p=0.03; rs2231142: OR 0.67, 95% CI 0.50–0.98, p=0.03) of a similar magnitude as those of our study. In the smaller SF1 study neither of the SNPs showed any significance with the disease risk. We also performed a meta-analysis between the three studies. A significant association with decreased CLL risk was observed for the non-synonymous rs2231142 SNP (OR=0.74, 95% CI 0.55–0.99, p=0.04). Figure 1 shows the forest plot of the individual study results and the combined estimate for rs2231142. Supplementary figure 1 illustrates the results for the remaining SNPs included in the meta-analysis.

Haplotypes and interaction analysis

Haplotype analysis was performed for each gene in the study. Two *ABCG2* haplotypes showed a study-wise significant association. Haplotype (rs13120400-rs1481012-rs2725256-rs2231142-rs1564481: A_A_A_C_G) (OR=0.78; 95%CI 0.67–0.91; *p*=0.001) and Haplotype (rs13120400-rs1481012-rs2725256-rs2231142-rs1564481: A_g_A_a_G) (OR=0.70; 95%CI 0.56–0.87; *p*=0.002) were associated with a decreased risk of B-NHL. None of the other haplotypes showed a study-wise statistically significant association with lymphoma risk, although haplotype (rs11917714-rs6785049-rs3732360-rs1054190: a_g_g_G) of the *NR1I2* gene showed a suggestive association with increased risk of B-NHL, which however did not reach study-wise significance (OR=1.22; 95%CI 1.05–1.43; *p*=0.009). Table 3 shows the distribution of the *ABCG2* and *NR1I2* haplotypes and their associations with lymphoma risk. Online Supplementary Table S3 shows the haplotypes of each gene and their association with lymphoma risk. The structure of the haplotype blocks of *ABCG2* is presented in Figure 2. Online Supplementary Figure 2 shows the LD blocks of the *ABCB1*, *ABCC2* and *NRI12* genes.

Furthermore we analyzed all the possible pair-wise interactions between SNPs using the MDR method. No interactions between genes emerged with a study-wise significant *p*-value.

Discussion

Detoxification of xenobiotics, including toxins, carcinogens, and drugs, is the main task of many metabolizing enzymes. Transporter proteins are centrally involved in xenobiotic defence. Export pumps are the gatekeepers for all cells and organelles, controlling uptake and efflux of crucial compounds such as sugars, amino acids, nucleotides, inorganic ions and drugs and reducing the local cellular burden of toxic compounds giving the individual cell protection against toxic effects. The importance of the genes that code for these proteins is underlined by the fact that it is generally assumed that at least 5% (>2,000) of all human genes are transport-related. [14]

In this study, we thoroughly captured common genetic variation across *ABCB1*, *ABCC2*, *ABCG2* and *NR1I2* genes and, to our knowledge, this is the most comprehensive evaluation of common variation in this crucial biological mechanism in relation with lymphoma risk. The intensive SNP tagging approach used provides a close to exhaustive analysis of associations of lymphoma risk with common polymorphic variants known for each of the loci studied. Moreover the analyses of haplotypes and gene-gene interactions give a comprehensive picture of the common genetic variability of the 4 genes in relation with HL, B-NHL and its more common subgroups. In this study we had sufficient power (over 0.80 for a codominant model) to detect OR=1.29 at alpha=0.001 (study-wise significance *p*-threshold) for a SNP with a MAF of 0.30 when considering all the study population as well as the B-NHL subgroup. For analyses in the HL sample set the power to detect an OR=1.5 for a SNP with a MAF of 0.30 was 0.80 for the codominant genetic model.

ABCG2 expression and its polymorphisms have been found, alone or in combination with polymorphisms of other transporters, to be associated with risk or prognosis of various malignant diseases and hematological diseases. [13, 24, 37–40] We found three SNPs (rs6857600, rs2231142 and rs1481012) on the *ABCG2* locus associated with risk of B-NHL overall, and with CLL in particular. One of these polymorphisms, rs6857600, emerged at study-wise level of significance threshold of p=0.001 with a decreased risk of B-NHL. This association was consistent also in the CLL subgroup although the association was not statistically significant after correction for multiple testing (p=0.004).

Two SNPs, rs1481012 and rs2231142, emerged at study-wise significance threshold of p=0.001 with a decreased risk of CLL, (p=0.0005 for rs1481012 and p=0.0004 for rs2231142). These two polymorphisms are in close linkage disequilibrium with each other (r^2=0.92) and therefore they have to be considered as a single association. To validate our strongest associations (rs11917714, rs1481012, rs15644814, rs2231142, rs2725256, rs6857600) we used the data from 2 recent GWAS [7, 32] and performed a meta-analysis. We observed that the rs2231142 SNP showed a statistically significant association (p=0.04) in the meta-analysis. On the other hand, rs1481012 did not show a statistically significant association with CLL risk in the meta-analysis, although the ORs and 95% CI calculated within the CLL GWAS (Mayo-GEC) were very similar to those ascertained in the EpiLymph study, while the results of the SF1 were different. It is worth mentioning that this polymorphism (rs1481012) was imputed in SF1 study and genotyped in the Mayo-GEC population, moreover the power to detect the association observed in the EpiLymph population was limited in the SF1 sample (67%). Hu and colleagues (2007) found that carriers of the A allele of the SNP rs2231142 run an increased risk of diffuse large B-cell lymphoma (DLBCL) in a Chinese population. [24] In our study we found that carriers of C allele of rs2231142 were associated with decreased risk of CLL and B-NHL, although they were not associated with DLBCL risk.

The reason of this discrepancy in the findings may be due to various factors, including the different ethnicity of the two populations, different environmental risk factors or the difference in the sample size of the two studies, the Chinese study being rather small compared to ours.

SNP rs2231142, which has been associated with risk of various diseases [41–44], lies in the fifth exon of the *ABCG2* gene and is a non synonymous SNP which results from an amino acid change from glutamine to lysine. This nonsynonymous substitution has been proposed by several studies to affect ABCG2 protein expression, membrane surface translocation, efflux activity, or ATPase activity. [30, 45–48] The majority of these reports point to the possible decreased activity of the mutant (A) allele. Therefore, our finding that the polymorphism is associated with decreased risk would seem to be inconsistent with the

functional evidence. Nevertheless it is worth noting that the results of these studies vary, showing inconsistencies in the effect of this polymorphism on ABCG2 protein activity. Additionally, several of the functional studies were performed in cell lines and therefore might not reflect the physiological situation in the organism.

None of the SNPs emerged as significant in this report were listed among the most significant results of the three recently conducted genome wide association studies (GWAS) on CLL. [32, 49, 50] We also analyzed haplotypes and SNP-SNP interactions, but the results did not explain more of the genetic susceptibility to the disease than the three SNPs alone. In this report we focused our attention on 4 genes where profound *a priori* knowledge of the influence of genetic variability on protein function exists. However other players (such as *ABCC1* and *ABCC3*) were left out and we cannot exclude possible interaction between the latter ones and the *ABCB1, ABCC2, ABCG2* and *NR1I2* genes. In conclusion, we have found strong associations between three common variants of the *ABCG2* gene, which has an important role in the elimination process of toxic agents in the organism, and the risk of B-NHL and CLL. We replicated the association of rs2231142 SNP in an independent population and after performing a meta-analysis the association remained statistically significant. Considering the three populations together, results of 939 cases and 2242 controls are presented, making it the largest investigation on genetic susceptibility of CLL. As transporter and well as metabolic genes affect risk of lymphoma only in presence of their carcinogenic substrate, further analyses shall be conducted to test the interaction between such substrates and the transporter and metabolic gene polymorphisms. This finding if further replicated may be a useful tool to identify a sub-group of individuals with an increased risk of CLL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ABC	ATP-binding cassette
MDR1	multidrug resistance 1

MRP2	multidrug resistance protein 2
BCRP	breast cancer resistance protein
NR1I2	nuclear receptor 1I2
PXR	pregnane X receptor
SNP	single nucleotide polymorphism
WHO	World Health Organization
HL	Hodgkin's Lymphoma
T-NHL	T-cell non-Hodgkin Lymphoma
B-NHL	B-cell non-Hodgkin Lymphoma
DLBCL	diffuse large B-cell lymphoma
FL	follicular lymphoma
CLL	chronic lymphocytic leukemia
MZL	marginal zone B-cell lymphoma
MCL	mantle cell lymphoma
MAF	minor allele frequency
LD	linkage disequilibrium
HWE	Hardy-Weinberg Equilibrium
MDR	Multifactor Dimensionality Reduction
OR	odds ratio
95%CI	95% confidence interval
GWAS	genome wide association study

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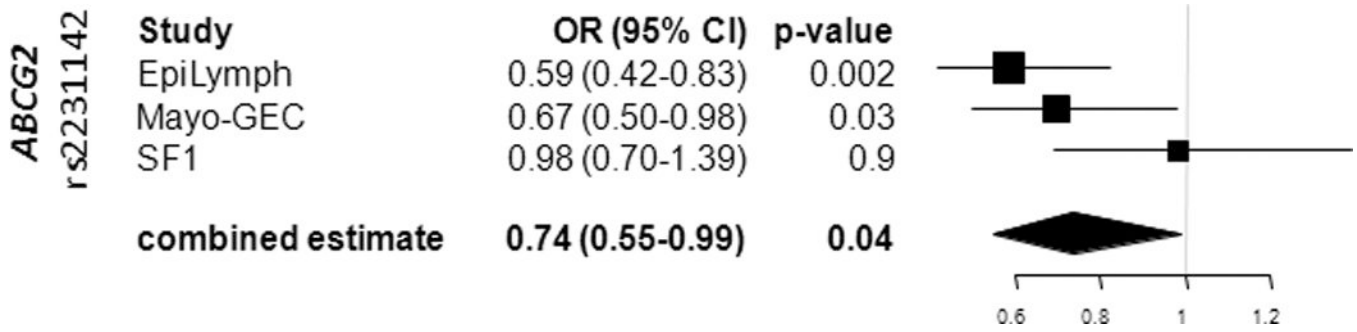

Figure 1. Association between rs2231142 *(ABCG2)* and CLL risk

Relative samples sizes are represented by size of symbols. Horizontal lines indicate the 95% confidence intervals for the respective allelic odds. The combined estimate is indicated by a diamond. For the Mayo-GEC population the OR calculation was based on imputed information.

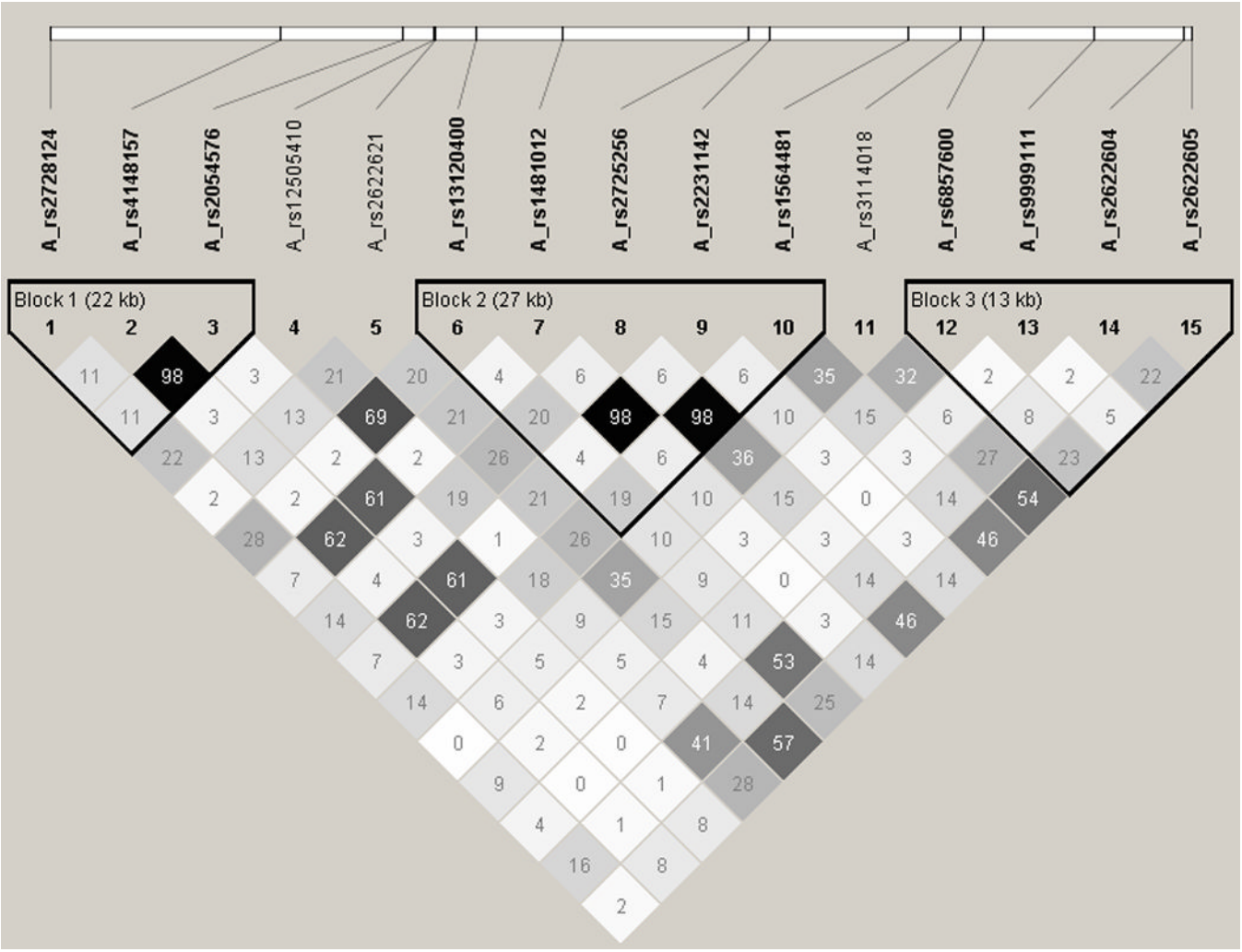

Figure 2. Haplotype blocks of the ABCG2 gene

Figure 2 shoes the estimates of the correlation coefficient r^2 in the ABCG2 gene, derived from the genotypes in the underlying study population in the Haploview software[28]. The LD relationship between each pair of SNP is indicated by the values in the squares. The shading indicates the extent of LD, a greater LD is represented by darker shading.

The rs1481012 and rs2231142 are in high LD (r^2=0.989), and the rs2775256 is in high LD with the rs1564481 (r^2=0.982).

Table 1

Characteristics of the study population.

Variable	Cases	Control subjects
	N (%)*	N (%)*
Age		
<30	147 (10)	129 (9)
30–39	147 (10)	153 (11)
40–49	175 (12)	183 (13)
50–59	269 (19)	274 (19)
60–69	390 (28)	397 (27)
=70	286 (21)	311 (21)
Study Centre		
Spain	301 (21)	318 (22)
France	149 (11)	152 (10)
Germany	518 (37)	530 (37)
Italy	106 (7)	110 (8)
Ireland	105 (7)	102 (7)
Czech Republic	235 (17)	235 (16)
Sex		
Male	787 (56)	802 (55)
Female	627 (44)	645 (45)
Lymphoma subtype		
Hodgkin lymphoma (HL)	259 (18)	-
T-cell lymphoma (T-NHL)	90 (6)	-
B-cell lymphoma (B-NHL)	1114 (76)	-
Diffuse large B-cell lymphoma (DLBCL)	391 (28)	-
Follicular lymphoma (FL)	195 (14)	-
Chronic lymphocytic leukaemia (CLL)	321 (23)	-
Mantle cell lymphoma (MCL)	48 (3)	-
Marginal zone B-cell lymphoma (MZL)	110 (8)	-
Removed samples†	67	44
	1481	1491

*Percentage is based on the final population used for statistical analysis.

†65 cases and 44 controls were removed due to low performance of the genotyping (call-rate below 0.95); additionally, 2 cases were excluded due to missing information on age.

Table 2

Odds Ratios (OR) and 95% Confidence Intervals (CI) for transporter gene related SNPs with B-NHL, HL and CLL in samples of the Epilymph study. Only SNPs with at least an association showing $p<0.01$ are presented. Significant associations after correction for multiple testing ($p\leq0.001$) are highlighted in bold).

Genotype	Controls*	B-NHL			CLL			Controls*	HL		
	N (%)	N (%)	OR† (95% CI)	*p*	N (%)	OR (95%CI)	*p*	N (%)	N (%)	OR (95%CI)	*p*
ABCC2 rs3740067											
CC	450 (38)	418 (39)	Reference		129 (40)	Reference		417 (36)	115 (44)	Reference	
CG	562 (47)	493 (46)	0.95 (0.79–1.13)	0.5	145 (45)	0.90 (0.68–1.18)	0.4	569 (49)	105 (41)	0.63 (0.46–0.87)	0.004
GG	183 (15)	151 (14)	0.90 (0.69–1.16)	0.4	46 (14)	0.89 (0.60–1.31)	0.6	170 (5)	39 (15)	0.7 (0.45–1.09)	0.1
CG/GG			0.93 (0.79–1.11)	0.4		0.90 (0.69–1.16)	0.4			0.65 (0.48–0.87)	0.004
			p-trend	0.4		*p*-trend	0.5			*p*-trend	0.02
ABCG2 rs1481012											
AA	959 (80)	902 (85)	Reference		284 (88)	Reference		923 (80)	224 (86)	Reference	
AG	228 (19)	155 (15)	0.71 (0.57–0.90)	0.004	33 (10)	0.50 (0.33–0.74)	**0.0005**	220 (19)	33 (13)	0.61 (0.40–0.93)	0.02
GG	9 (1)	10 (1)	1.15 (0.47–2.86)	0.8	4 (1)	1.57 (0.47–5.26)	0.5	13 (1)	2 (1)	0.51 (0.10–2.53)	0.4
AG/GG			0.73 (0.59–0.91)	0.005		0.54 (0.37–0.78)	**0.001**			0.60 (0.40–0.91)	0.02
			p-trend	0.01		*p*-trend	0.005			*p*-trend	0.02
ABCG2 rs1564481											
GG	501 (42)	407 (38)	Reference		121 (38)	Reference		473 (41)	95 (37)	Reference	
GA	564 (47)	496 (46)	1.08 (0.90–1.29)	0.4	145 (45)	1.08 (0.82–1.42)	0.6	554 (48)	128 (49)	1.26 (0.92–1.74)	0.2
AA	131 (11)	164 (15)	1.54 (1.18–2.00)	0.002	55 (17)	1.85 (1.26–2.71)	0.002	129 (11)	36 (14)	1.43 (0.90–2.28)	0.1
GA/AA			1.17 (0.98–1.38)	0.08		1.22 (0.94–1.58)	0.1			1.30 (0.96–1.75)	0.09
			p-trend	0.002		*p*-trend	0.007			*p*-trend	0.08
ABCG2 rs2231142											
CC	957 (80)	898 (84)	Reference		284 (88)	Reference		921 (80)	224 (86)	Reference	
CA	229 (19)	158 (15)	0.73 (0.58–0.91)	0.005	33 (10)	0.49 (0.33–0.73)	**0.0004**	221 (19)	33 (13)	0.60 (0.40–0.92)	0.02
AA	10 (1)	11 (1)	1.15 (0.49–2.74)	0.7	4 (1)	1.42 (0.43–4.65)	0.6	14 (1)	2 (1)	0.49 (0.10–2.43)	0.4

Genotype	Controls*	B-NHL			CLL			Controls*	HL		
	N (%)	N (%)	OR† (95% CI)	*p*	N (%)	OR (95%CI)	*p*	N (%)	N (%)	OR (95%CI)	*p*
CA/AA			0.75 (0.60–0.93)	0.009		0.53 (0.36–0.77)	**0.001**			0.59 (0.39–0.90)	0.01
			p-trend	0.02		*p*-trend	0.004			*p*-trend	0.01
ABCG2 rs2725256											
AA	498 (42)	403 (38)	Reference		119 (37)	Reference		469 (41)	94 (36)	Reference	
AG	562 (47)	496 (46)	1.09 (0.91–1.30)	0.4	146 (45)	1.10 (0.84–1.45)	0.5	555 (48)	129 (50)	1.29 (0.94–1.77)	0.1
GG	136 (11)	168 (16)	1.52 (1.17–1.98)	0.002	56 (17)	1.82 (1.25–2.66)	0.002	132 (11)	36 (14)	1.40 (0.88–2.23)	0.2
AG/GG			1.17 (0.99–1.39)	0.07		1.24 (0.96–1.60)	0.10			1.31 (0.97–1.77)	0.08
			p-trend	0.005		*p*-trend	0.007			*p*-trend	0.08
ABCG2 rs6857600											
GG	696 (58)	693 (65)	Reference		211 (66)	Reference		681 (59)	153 (59)	Reference	
GA	421 (35)	319 (30)	0.76 (0.64–0.91)	0.003	86 (27)	0.63 (0.47–0.84)	0.002	409 (35)	84 (32)	0.94 (0.69–1.30)	0.7
AA	79 (7)	55 (5)	0.71 (0.49–1.01)	0.06	24 (7)	0.94 (0.57–1.55)	0.8	66 (6)	22 (8)	1.37 (0.77–2.42)	0.3
GA/AA			0.75 (0.64–0.90)	**0.001**		0.68 (0.52–0.88)	0.004			1.01 (0.75–1.36)	1.0
			p-trend	0.002		*p*-trend	0.03			*p*-trend	0.6
NR1I2 rs11917714											
GG	822 (69)	684 (64)	Reference		215 (67)	Reference		800 (69)	164 (63)	Reference	
GA	333 (28)	327 (31)	1.18 (0.98–1.42)	0.07	91 (28)	1.03 (0.78–1.36)	0.9	319 (28)	82 (32)	1.29 (0.94–1.79)	0.1
AA	40 (3)	55 (5)	1.65 (1.08–2.51)	0.02	15 (5)	1.44 (0.77–2.70)	0.3	37 (3)	13 (5)	1.35 (0.67–2.76)	0.4
GA/AA			1.23 (1.03–1.47)	0.02		1.07 (0.82–1.40)	0.6			1.30 (0.96–1.77)	0.09
			p-trend	0.007		*p*-trend	0.4			*p*-trend	0.1

*1196 controls were taken into account for the risk analysis with the B-cell lymphoma cases and 1153 controls were taken into account for the risk analysis with the Hodgkin lymphoma cases.

†Odds Ratios were adjusted for the matching variables: age (continuous), sex and study center (categorical); the common homozygote was used as reference group for each SNP.

Table 3

Odds Ratios and 95% Confidence Intervals for estimated Haplotypes of the *ABCG2* and *NR1I2* genes associated with B-NHL and subtypes.

			B-NHL			DLBCL			FL			CLL		
Gene	**Structure**	**Freq.**	**OR***	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
ABCG2	Block2†													
	A-A-g-C-a	0.36	Reference			Reference			Reference			Reference		
	g-A-A-C-G	0.28	0.95	(0.82–1.10)	0.5	0.99	(0.80–1.21)	0.9	0.93	(0.70–1.22)	0.6	0.89	(0.71–1.11)	0.3
	A-A-A-C-G	0.25	**0.78**	**(0.67–0.91)**	**0.001**	0.80	(0.64–0.99)	0.04	0.79	(0.60–1.05)	0.10	**0.74**	**(0.59–0.94)**	**0.01**
	A-g-A-a-G	0.09	**0.70**	**(0.56–0.87)**	**0.002**	**0.70**	**(0.51–0.96)**	**0.03**	0.86	(0.58–1.28)	0.5	**0.53**	**(0.37–0.77)**	**0.001**
NR1I2	Block2‡													
	G-A-A-G	0.60	Reference			Reference			Reference			Reference		
	a-g-g-G	0.19	**1.22**	**(1.05–1.43)**	**0.009**	**1.26**	**(1.03–1.55)**	**0.03**	**1.39**	**(1.06–1.83)**	**0.02**	1.13	(0.90–1.43)	0.3
	G-g-A-a	0.13	1.09	(0.90–1.30)	0.4	0.90	(0.69–1.18)	0.4	1.28	(0.92–1.77)	0.1	1.20	(0.91–1.57)	0.2
	G-g-g-G	0.08	0.82	(0.66–1.01)	0.07	0.83	(0.62–1.13)	0.2	**0.56**	**(0.34–0.91)**	**0.02**	0.97	(0.70–1.33)	0.8

Abbreviations are explained in Table 2.

*Odds Ratios were adjusted for the matching variables: age (continuous), sex and study center (categorical); the common haplotype was used as reference group.

†SNP order: rs13120400-rs1481012-rs2725256-rs2231142-rs1564481. Uppercase letters indicate major allele, lowercase letters denote minor allele

‡SNP order: rs11917714-rs6785049-rs3732360-rs1054190. Uppercase letters indicate major allele, lowercase letters denote minor allele