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ORIGINAL ARTICLE

Influence of polymorphisms in ERCC5, XPA and MTR DNA repair and synthesis genes in B-cell lymphoma risk. A case-control study in Spanish population

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Summary

Purpose: *Functions pertaining to DNA repair and synthesis are believed to play a critical role in cancer development and seem to be affected by genetic polymorphisms. Herein we performed a case-control study evaluating the influence of three single nucleotide polymorphisms (SNPs) in XPA, ERCC5 and MTR [rs1800975 (G-4A), rs17655 (Asp1104His) and rs1805087 (A2756G), respectively] in lymphoma risk.*

Methods: *Genotype distributions were studied in 213 lymphoma Caucasian patients (193 non-Hodgkin/NHL and 20 Hodgkin lymphoma/HL) and 214 controls, residents in a region of Southeast Spain.*

Results: *No significant differences were observed in the genotype distributions between cases and controls for the studied SNPs. This lack of association was also observed when stratifying for gender or lymphoma type.*

Conclusion: *Our results suggest that the rs1800975, rs17655 and rs1805087 SNPs in DNA repair and synthesis of genes do not seem to play a major role in lymphoma susceptibility.*

Key words: *ERCC5, lymphoma, MTR, polymorphism, XPA, XPG*

Introduction

XPA (Xeroderma pigmentosum complementation group A; also known as XPI or XPAC) and ERCC5 (Excision repair cross-complementing rodent repair deficiency, complementation group 5, also known as XPG or XPGC) are two enzymes part of the nucleotide excision repair (NER) pathway implicated in DNA repair machinery whose genes are located in 9q22.3 and 13q33, respectively. Qualitative and/or quantitative alterations in the activity of such enzymes can lead to abnormal accumulation of DNA mutations which eventually can trigger a carcinogenic process. MTR (5-methyltetrahydrofolate-homocysteine methyltransferase, also known as MS) whose gene is located in 1q43 is an enzyme involved in folate and methionine metabolism playing an essential role in both DNA synthesis and methylation processes. Similarly, suboptimal function of such enzymes would also lead to higher rate of DNA mutations [1].

In fact, SNPs affecting ERCC5, XPA and MTR genes have been found to influence the susceptibility to the development of certain cancer types. Although, several studies have reported the association of certain allelic variants in XPA, ERCC5 and MTR such as rs1800975 (also known as G-4A), rs17655 (Asp1104His) and rs1805087 (A2756G) with increased risk of breast [2,3], lung [4,5], prostate [6] or head and neck [7] cancers, little information has been reported in lymphoma [8-10].

It has been demonstrated that exposure to UV and chemical industry compounds can cause DNA damage [11,12]. Under these circumstances the effect of SNPs in DNA repair genes or in genes involved in DNA synthesis may be of special relevance for the appearance of inter-individual dif-

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ferences in cancer susceptibility. In this study we aimed at studying the influence of three SNPs in XPA, ERCC5 and MTR (rs1800975, rs17655 and rs1805087, respectively) in lymphoma by means of a case-control study.

Methods

Study subjects

Blood samples were obtained from 213 B-cell lymphoma (BCL) Caucasian patients -193 NHL and 20 HL- residents of either Murcia or Cartagena, in the Southeast of Spain. Our study subjects came largely from a previous described series of patients diagnosed between 2004 and 2010 at the HUSMR in Cartagena and Morales Meseguer University Hospital in Murcia [13]. Data on gender, age and place of residence were obtained from medical records. The control group comprised 214 unrelated Caucasian healthy blood donors matched for age, gender and geographical location without a previous history of malignancy. Informed consent was obtained from all subjects, the study was approved by the local Ethics Committee and was in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

DNA extraction and genotyping

Total genomic DNA was obtained from blood samples using the automatic DNA extraction system Maxwell 16 and DNA extraction kit for blood samples (cat: AS1010) (Promega, Madison, USA) according to the manufacturer´s instructions. DNA was quantified by UV absorbance using the Biophotometer by Eppendorf (Hilden, Germany).

Polymorphisms were determined by allelic discrimination using TaqMan probes and a 7500F real-time PCR thermocycler both provided by Applied Biosystems (Foster City, CA).

Statistics

Statistical analysis was performed using the SPSS computer program Version 15.0 (Chicago, Illinois, USA). Student´s t-test was used to compare mean age of cases and controls and Yeats-corrected Pearson x^2 test was used to evaluate statistical significance of genotype distribution in cases and controls using the dominant model for the p-value calculation which was considered taking into account the Bonferroni correction for multiple testing, thus taking $p < 0.017$ as significant.

Results

Mean age did not differ between cases and controls (mean 53.7; ±SD 15.1 vs. 53.9±8.4; p=0.866). Forty-nine percent (N=104) of cases were female whereas this percentage was 53.3% (N=114) in controls (p=0.358). Genotyping was successfully performed on all study subjects. The genotype frequencies in the study population were XPA: GG 42.6%, GA 50.1%, AA 7.3%; ERCC5: GG 57.1%, GC 35.6%, CC 7.3% and MTR: AA 70.2%, AG 25.1%, GG 4.7%, similar to the HapMap-CEU (European) frequencies (XPA: GG 37.2%, GA 49.6%, AA 13.3%; ERCC5: GG 53.3%, GC 40.0%, CC 6.7% and MTR: AA 68.8%, AG 30.3%, GG 9.0%; [http://hapmap.ncbi.nlm.nih.gov].

No significant differences were observed in the genotype distributions of rs1800975, rs17655 and rs1805087 polymorphisms between cases and controls (Table 1). This lack of association was

Table 1. *XPA*, *ERCC5* and *MTR* genotype distributions in cases and controls and according to gender. P-value was calculated using the dominant model

		XPA (GG vs GG+GA)			*ERCC5 (GG vs GC+CC)*			*MTR (AA vs AG+ GG)*		
		GG N (%)	*GA N (%)*	*AA N (%)*	*GG N (%)*	*GC N (%)*	*CC N (%)*	*AA N (%)*	*AG N (%)*	*GG N (%)*
Total	Cases	90 (42.3)	102 (47.9)	21 (9.9)	125 (58.7)	71 (33.3)	17 (8.0)	148 (69.8)	52 (24.5)	12 (5.7)
	Controls	92 (43.0)	112 (52.3)	10 (4.7)	119 (55.6)	81 (37.9)	14 (6.5)	151 (70.6)	55 (25.7)	8 (3.7)
	p (OR; 95%CI)	*0.878 (1.0; 0.7-1.4)*			*0.521 (1.1; 0.8-1.7)*			*0.866 (1.0; 0.6-1.5)*		
Females	Cases	40 (38.5)	54 (51.9)	10 (9.6)	65 (63.1)	34 (33.0)	4 (3.9)	70 (68.6)	26 (25.5)	6 (5.9)
	Controls	47 (41.2)	61 (53.5)	6 (5.3)	65 (57.0)	40 (35.1)	9 (7.9)	83 (72.8)	27 (23.7)	4 (3.5)
	p (OR; 95%CI)	*0.677 (0.9; 0.5-1.5)*			*0.361 (1.3; 0.7-2.2)*			*0.500 (0.8; 0.5-1.5)*		
Males	Cases	50 (45.9)	48 (44.0)	11 (10.1)	60 (45.5)	37 (33.6)	13 (11.8)	78 (70.9)	26 (23.6)	6 (5.5)
	Controls	45 (45.0)	51 (51.0)	4 (4.0)	54 (54.0)	41 (41.0)	5 (5.0)	68 (68.0)	28 (28.0)	4 (4.0)
	p (OR; 95%CI)	*0.512; (0.8; 0.5-1.5)*			*0.937 (1.0; 0.6-1.8)*			*0.647 (1.1; 0.6-2.1)*		

CI:confidence interval, OR:odds ratio. For other abbreviations see text

also observed when stratifying for gender or lymphoma type (Table 2).

Discussion

Potential carcinogenic exposure of either endogenous or exogenous nature can cause DNA damage which, in turn, can contribute to cancer development. Therefore, the efficiency of DNA repair and synthesis enzymatic machinery is critical for diminishing the carcinogenic effects. Polymorphisms in the genes coding these enzymes are believed to constitute a molecular basis for inter-individual differences in cancer susceptibility. Herein, we have studied the effect of rs1800975, rs17655 and rs1805087 polymorphisms in XPA, ERCC5 and MTR genes, respectively, in lymphoma risk. These polymorphisms have demonstrated a susceptibility role in different types of tumors [2,4,14], however their role in lymphoma is unknown or controversial. Overall, genetic polymorphisms in NER pathway seem to be associated with NHL [8] and several studies have found some associations between polymorphisms in XRCC1 DNA repair gene and lymphoma risk [8,15-17] whilst others did not find such a relationship [18-20].

XPA is responsible for repair of UV radiation-induced photoproducts and DNA adducts induced by chemical carcinogens. Wang et al. demonstrated that the rs1800975 XPA polymorphism was associated with differences in DNA damage in coke oven workers [21]. Despite this fact, our results did not reveal any association of this polymorphism with lymphoma, in concordance with the results reported by El-Zein et al. for HL [19].

ERCC5 is a single-strand specific DNA endonuclease that makes the 3' incision in DNA excision repair following UV-induced damage. El-Zein et al. in HL [19] and Shen in NHL [8] did not find any relationship between ERCC5 rs17655 and lymphoma risk, although the latter group found an association with NHL in American women [20]. Accordingly to the former studies we did not observed any relationship between this polymorphism and lymphoma susceptibility in our study population, either in males or females.

MTR catalyzes the final step in methionine biosynthesis and is involved in both DNA synthesis and methylation. Although a meta-analysis demonstrated an effect of MTR rs180587 on NHL development [22] these authors and others did not find such an association in the study population of NHLs [22-24] or lymphomas [9]. In accordance with these studies, we did not identify any relationship between this SNP and lymphoma susceptibility in our study. In contrast, the study by Lincz et al. showed a protective role of AG/GG genotypes in NHL risk [10]. However, in this work cancer tissue specimens were used for SNP genotyping which is not recommended for genetic susceptibility studies because possible allelic somatic losses produced during the carcinogenic

Table 2. *XPA, ERCC5* and *MTR* genotype distributions according to the lymphoma diagnosis. P-value was calculated using the dominant level

	XPA			*ERCC5*			*MTR*		
	GG N(%)	*GA* N(%)	*AA* N(%)	*GG* N(%)	*GC* N(%)	*CC* N(%)	*AA* N(%)	*AG* N(%)	*GG* N(%)
Controls	92 (43.0)	112 (52.3)	10 (4.7)	119 (55.6)	81 (37.9)	14 (6.5)	151 (70.6)	55 (25.7)	8 (3.7)
NHL	86 (44.6)	91 (47.2)	16 (8.3)	118 (61.1)	62 (32.1)	13 (6.7)	135 (70.3)	48 (25.0)	9 (4.7)
p (OR; 95%CI)	*0.750 (1.1; 0.7-1.6)*			*0.228 (1.3; 0.9-2.0)*			*0.956 (1.0; 0.6-1.5)*		
FL	56 (49.6)	56 (41.5)	12 (8.9)	81 (60.0)	46 (34.1)	8 (5.9)	90 (67.2)	36 (26.9)	8 (6.0)
p (OR; 95%CI)	*0.698 (1.1; 0.7-1.7)*			*0.884 (1.0; 0.7-1.6)*			*0.504 (0.9; 0.5-1.4)*		
DLBCL	11 (32.4)	20 (58.8)	3 (8.8)	21 (61.8)	8 (23.5)	5 (14.7)	28 (82.4)	6 (17.6)	0 (0)
p (OR; 95%CI)	*0.242 (0.6; 0.3-1.4)*			*0.778 (1.1; 0.5-2.3)*			*0.154 (1.9; 0.8-4.9)*		
HL	4 (20.0)	11 (55.0)	5 (25.0)	7 (35.0)	9 (45.0)	4 (20.0)	13 (65.0)	4 (20.0)	4 (15.0)
p (OR; 95%CI)	*0.046 (0.3; 0.1-1.0)*			*0.037 (0.4; 0.1-1.0)*			*0.410 (0.7; 0.3-1.7)*		

NHL: non-Hodgkin´s lymphoma, HL: Hodgkin´s lymphoma, FL: follicular lymphoma, DLBCL: diffuse large B-cell lymphoma, CI: confidence interval, OR: odds ratio. For other abbreviations see text

process may mask the germline genotype. Interestingly, Niclot et al. after studying the rs180587 genotype distribution in 172 follicular lymphoma (FL) cases and 206 controls found that individuals carrying the AA genotype had 2-fold higher risk of FL [25]. We did not observe this relationship either in our 134 FL cases.

The effect of polymorphisms in DNA repair and synthesis genes in cancer risk is dependent on the particular carcinogenic process, thus it is subjected to particular environmental factors and other genetic determinants. This may be the reason for the inconsistency observed in these studies. In any case, our results suggest that the rs1800975, rs17655 and rs1805087 polymorphisms in XPA, ERCC5 and MTR genes do not seem to play a major role in lymphoma susceptibility.

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References

1. Zingg JM, Jones PA. Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. Carcinogenesis 1997; 18: 869-882.
2. Han W, Kim KY, Yang SJ, Noh DY, Kang D, Kwack K. SNP-SNP interactions between DNA repair genes were associated with breast cancer risk in a Korean population. Cancer 2012; 118: 594-602.
3. de Cássia Carvalho Barbosa R, da Costa DM, Cordeiro DE, Vieira AP, Rabenhorst SH. Interaction of MTHFR C677T and A1298C, and MTR A2756G gene polymorphisms in breast cancer risk in a population in Northeast Brazil. Anticancer Res 2012; 32: 4805-4811.
4. Qian B, Zhang H, Zhang L, Zhou X, Yu H, Chen K. Association of genetic polymorphisms in DNA repair pathway genes with non-small cell lung cancer risk. Lung Cancer 2011; 73: 138-146.
5. Jeon HS, Kim KM, Park SH et al. Relationship between XPG codon 1104 polymorphism and risk of primary lung cancer. Carcinogenesis 2003; 24: 1677-1681.
6. Berhane N, Sobti RC, Mahdi SA. DNA repair genes polymorphism (XPG and XRCC1) and association of prostate cancer in a north Indian population. Mol Biol Rep 2012; 39: 2471-2479.
7. Galbiatti AL, da Silva LM, Ruiz-Cintra MT et al. Association between 11 genetic polymorphisms in folate-metabolising genes and head and neck cancer risk. Eur J Cancer 2012; 48: 1525-1531.
8. Shen M, Purdue MP, Kricker A et al. Polymorphisms in DNA repair genes and risk of non-Hodgkin's lymphoma in New South Wales, Australia. Haematologica 2007; 92: 1180-1185.
9. Matsuo K, Hamajima N, Suzuki R et al. Methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms and reduced risk of malignant lymphoma. Am J Hematol 2004; 77: 351-357.
10. Lincz LF, Scorgie FE, Kerridge I, Potts R, Spencer A, Enno A. Methionine synthase genetic polymorphism MS A2756G alters susceptibility to follicular but not diffuse large B-cell non-Hodgkin's lymphoma or multiple myeloma. Br J Haematol 2003; 120: 1051-1054.
11. Skipper PL, Kim MY, Sun HL, Wogan GN, Tannenbaum SR. Monocyclic aromatic amines as potential human carcinogens: old is new again. Carcinogenesis 2010; 31: 50-58.
12. Stone MP, Huang H, Brown KL, Shanmugam G. Chemistry and structural biology of DNA damage and biological consequences. Chem Biodivers 2011; 8: 1571-1615.
13. Ruiz-Cosano J, Conesa-Zamora P, González-Conejero R et al. Role of GSTT1 and M1 null genotypes as risk factors for B-cell lymphoma: influence of geographical factors and occupational exposure. Mol Carcinog 2012; 51: 508-513.
14. Cho S, Kim MJ, Choi YY et al. Associations between polymorphisms in DNA repair genes and TP53 mutations in non-small cell lung cancer. Lung Cancer 2011; 73: 25-31.
15. Kim IS, Kim DC, Kim HG et al. DNA repair gene XRCC1 polymorphisms and haplotypes in diffuse large B-cell lymphoma in a Korean population. Cancer Genet Cytogenet 2010; 196: 31-37.
16. Liu J, Song B, Wang Z et al. DNA repair gene XRCC1 polymorphisms and non-Hodgkin lymphoma risk in a Chinese population. Cancer Genet Cytogenet 2009; 191: 67-72.
17. Worrillow L, Roman E, Adamson PJ, Kane E, Allan JM, Lightfoot TJ. Polymorphisms in the nucleotide excision repair gene ERCC2/XPD and risk of non-Hodgkin lymphoma. Cancer Epidemiol 2009; 33: 257-260.
18. Matsuo K, Hamajima N, Suzuki R et al. Lack of association between DNA base excision repair gene XRCC1 Gln399Arg polymorphism and risk of malignant lymphoma in Japan. Cancer Genet Cytogenet 2004; 149:

77-80.

19. El-Zein R, Monroy CM, Etzel CJ et al. Genetic polymorphisms in DNA repair genes as modulators of Hodgkin disease risk. Cancer 2009; 115: 1651-1659.
20. Shen M, Zheng T, Lan Q et al. Polymorphisms in DNA repair genes and risk of non-Hodgkin lymphoma among women in Connecticut. Hum Genet 2006; 119: 659-668.
21. Wang F, He Y, Guo H et al. Genetic variants of nucleotide excision repair genes are associated with DNA damage in coke oven workers. Cancer Epidemiol Biomarkers Prev 2010; 19: 211-218.
22. Weiner AS, Beresina OV, Voronina EN et al. Polymorphisms in folate-metabolizing genes and risk of non-Hodgkin's lymphoma. Leuk Res 2011; 35: 508-515.
23. Lee KM, Lan Q, Kricker A et al. One-carbon metabolism gene polymorphisms and risk of non-Hodgkin lymphoma in Australia. Hum Genet 2007; 122: 525-533.
24. Lightfoot TJ, Skibola CF, Willett EV et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev 2005; 14: 2999-3003.
25. Niclot S, Pruvot Q, Besson C et al. Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Blood 2006; 108: 278-285.