Check for updates

Brief report

Relationship between glutathione S-transferase M1, T1, and P1 polymorphisms and chronic lymphocytic leukemia

Martin Yuille, Alison Condie, Chantelle Hudson, Zsofia Kote-Jarai, Elaine Stone, Rosalind Eeles, Estella Matutes, Daniel Catovsky, and Richard Houlston

Interindividual differences in susceptibility to hematologic malignancies may be mediated in part through polymorphic variability in the bioactivation and detoxification of carcinogens. The glutathione S–transferases (GSTs) have been implicated as susceptibility genes in this context for a number of cancers. The aim of this study was to examine whether polymorphic variation in GSTs confers susceptibility to chronic lymphocytic leukemia (CLL). *GSTM1, GSTT1,* and *GSTP1* genotypes were determined in 138 patients and 280 healthy individuals. The frequency of both *GSTM1* and *GSTT1* null genotypes and the *GSTP1-Ile* allele was higher in cases than in controls. There was evidence of a trend in increasing risk with the number of putative "high-risk" alleles of the GST family carried ($P = .04$). The risk of CLL associated with possession of all 3 "high-risk" genotypes was increased 2.8-fold (OR = 2.8, 95% confidence interval: 1.1-6.9). Our findings suggest that heritable GST status may influence the risk of developing CLL. (Blood. 2002;99:4216-4218)

© 2002 by The American Society of Hematology

Introduction

B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia, accounting for around 30% of all cases.[1] There is increasing evidence that predisposition to CLL involves both inherited and environmental factors.[2,3] It is likely that part of the inherited susceptibility to CLL may be determined by interindividual differences in the bioactivation of procarcinogens and detoxification of carcinogens.

The glutathione S–transferases (GSTs) are a superfamily of genes whose products are phase II enzymes, catalyzing the conjugation of reactive intermediates to soluble glutathione.[4] GSTM1 and GSTP1 detoxify carcinogenic polycyclic aromatic hydrocarbons such as benzo-(a)pyrene, whereas GSTT1 is responsible for the detoxification of smaller reactive hydrocarbons, such as ethylene oxide.[4]

Differences in the activities of some GSTs are determined by genetic polymorphisms.[4] GSTM1 activity is absent in ~50% of whites as a consequence of the inheritance of 2 null alleles (deletion of the gene). Similarly, GSTT1 activity is deficient in ~20% of whites, resulting from homozygous deletion. The *GSTP* subfamily comprises only *GSTP1*. The 1578A>G substitution in *GSTP1* creates the *Ile105Val* polymorphism that leads to expression of an enzyme with reduced activity.[4]

There is epidemiologic evidence that exposure to aliphatic hydrocarbons and chlorinated hydrocarbons plays a role in the etiology of CLL.[3,5-8] This, coupled with the proposed role of GSTs in the etiology of a number of common cancers[9] provides a strong rationale for evaluating *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms as risk factors for CLL.

Study design

Patients

Blood samples were obtained from 138 white patients (62% male; 38% female; mean age at presentation 54 years, SD: 12) with B-cell CLL referred to the Royal Marsden Hospital NHS Trust. The diagnosis of CLL was based on standard hematologic and immunologic criteria. The proportion of patients with Binet stages A, B, and C were 58%, 14%, and 28%, respectively. Median white cell count in the cases was 22×10^9. Control blood samples were obtained from 280 geographically and ethnically matched individuals who were spouses of patients enrolled in another cancer study. None of these individuals had a personal or family history of malignancy. Venous blood samples were obtained with informed consent and ethical review board approval. DNA was salt extracted from ethylenediaminetetraacetic acid (EDTA) blood samples using a standard sucrose lysis method.

Genotyping

GST1 genotypes were determined by polymerase chain reaction (PCR) methods. The presence or deletion of *GSTM1* and *GSTT1* were determined using primer pairs 5′-CTG CCC TAC TTG ATT GAT GGG-3′, 5′-CTG GAT TGT AGC AGA TCA TGA-3′, and 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′, 5′-TCA CCG GAT CAT GGC CAG CA-3′, respectively. Interferon, alpha-5 (IFNA5) was used as an internal control. Homozygous nondeleted and heterozygous genotypes were not distinguished. *GSTP1-Ile105Val* genotypes were assigned by PCR–restriction fragment length polymorphism (RFLP) using primers 5′-ACC CCA GGG CTC TAT GGG AA -3′ and 5′-TGA GGG CAC AAG CCC CT-3′ and the restriction enzyme *Bsm*AI. PCR was undertaken using 25 ng genomic DNA in a 15l reaction mixture containing 1 mM $MgCl_2$, 6 pM of each primer, and 0.5 U Taq polymerase. PCR products were separated using 3.5% agarose gels.

Statistical analysis

The relationship between *GSTM1*, *GSTT1*, and *GSTP1* genotypes and risk of CLL was assessed by means of the odds ratio (OR) with 95% confidence limits calculated by logistic regression. *GSTM1* and *GSTT1* genotypes were classified as either null (homozygous deletion) or nondeleted. A test for trend (P_{trend}) in increasing the risk of CLL by having more than one putative high-risk allele or genotype was evaluated by means of the chi-square test.

From the Academic Department of Haematology and Cytogenetics, and the Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, United Kingdom.

Submitted May 21, 2001; accepted January 6, 2002.

Supported by grants from the Leukaemia Research Fund (London WCIN 3JJ) and the Kay Kendall Leukaemia Trust (London EC4A 3FF).

Reprints: R. S. Houlston, Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, United Kingdom SM2 5NG; e-mail: r.houlston@icr.ac.uk.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2002 by The American Society of Hematology

Downloaded from http://ashpublications.net/blood/article-pdf/99/11/4216/1685675/h8110200004216.pdf by guest on 02 June 2024

Table 1. Frequency of *GSTM1, GSTT1,* and *GSTP1* genotypes in CLL and controls

Polymorphism	Cases	Controls	Odds ratio	95% confidence interval	*P*
GSTM1	(n = 138)	(n = 270)			
Present	61	135	1		
Null	77	135	1.3	0.8-1.9	NS
GSTT1	(n = 138)	(n = 278)			
Present	97	212	1		
Null	41	66	1.4	0.9-2.2	NS
GSTP1	(n = 138)	(n = 273)			
Ile-Val	59	140	1		
Ile-Val	63	105	1.4	0.9-2.2	
Val-Val	16	28	1.4	0.7-2.7	P_{trend} = NS

The relationship between *GSTM1, GSTT1,* and *GSTP1* genotypes and stage and white blood count was assessed by Anova. Departure in the distribution of genotypes from Hardy-Weinberg equilibrium was assessed by means of the chi-square test. A *P* value of .05 was considered statistically significant. All computations were calculated using the statistical software package STATA, version 6.0 (Stata Corporation, College Station, TX).

Results and discussion

The frequency of the *GSTM1* and *GSTT1* null alleles in the controls were 50% (135/270) and 23% (66/270), respectively, which is in agreement with the previous documented findings in white populations.[4] The frequency of these genotypes in CLL was 56% (77/138) and 30% (41/138), respectively (Table 1). The distribution of *GSTP1* genotypes within cases and controls was not significantly different from that expected under Hardy-Weinberg equilibrium ($P = .9$ and .2, respectively). The frequencies of *GSTP1* heterozygotes and *GSTP1* homozygotes in controls were 38% (105/273) and 10% (28/273), respectively, also in agreement with previous estimates.[4] The frequencies of these genotypes were higher in the cases, 46% (63/138) and 12% (16/138), respectively (Table 1), but these differences did not attain formal statistical significance. Sex- and age-adjusted ORs were no different from crude ratios. In order to assess the existence of any interaction between the 3 GST genotypes we calculated the frequency of the simultaneous presence of the 3 putative "high-risk" genotypes. Individuals carrying all 3 low-risk genotypes—*GSTM1* and *GSTT1* nondeleted and *GSTP1-Ile105Ile*—served as the reference group. Heterozygotes and homozygotes for the *GSTP1-105Val* allele were combined for the analysis. Table 2 shows the risk of CLL associated with each combination of genotypes and the trend associated with 1, 2, and 3 putative high-risk genotypes. There was evidence of a trend of increasing risk with the number of high-risk GST alleles. The risk of CLL increased as the number of high-risk genotypes increased ($P_{trend} = .04$), and individuals harboring all 3 high-risk genotypes had a 2.8-fold increase in risk of CLL (95% confidence interval [CI]: 1.1-6.9). This suggests a possible synergistic effect between GST genotypes.

Allelic loss in cells used in genetic analyses is a potential source of bias, as genotyping assays do not always distinguish between homo- and heterozygote states. An apparent increase in *GSTM1* and *GSTT1* homozygotes may be due to loss of heterozygosity of peripheral leukocytes used for DNA extraction. If this is the case, a relationship between white blood count and GST status should be detectable. There was no evidence for an association between *GSTM1, GSTT1,* or *GSTP1* status and white blood count (*P* values .5, .7, and .4, respectively). The other potential source of bias is if a "case-case" effect is operating such that individuals with more advanced disease have a higher probability of having a "high-risk" allele. There was no evidence for such an effect as there was no relationship between *GSTM1, GSTT1,* or *GSTP1* status and stage (*P* values .2, .9, and 1.0, respectively).

Many studies have reported a relationship between GST variants and risk of a variety of common cancers including hematologic malignancies such as acute lymphoblastic and myeloid leukemia.[8] However, only one study has examined specifically the relationship between polymorphic variation in GSTs and CLL.[10] While this study failed to show a relationship between *GSTM1* status and CLL, it was only based on 13 cases and hence was severely underpowered to detect a relationship on the basis of the probable genotypic risk associated with any common low-risk allele. In our study we found that carrying more than one of the putative high-risk GST genotypes significantly increases the risk of developing CLL, the risk being highest with possession of all 3 high-risk genotypes. It is conceivable that these variants will interact with environmental carcinogens, and certain combinations will better define at-risk groups. Information about exposure to environmental carcinogens was, however, unfortunately not available from either the cases or controls in our study to examine this possibility. While the risk of CLL associated with GST genotypes may be small and further studies are required to validate our observations, the high population prevalence of these high-risk alleles means that heritable GST status may make a significant impact on CLL incidence.

Acknowledgments

We acknowledge the assistance of Benjamin Hilditch and Andrea Marossy in the ascertainment and collection of patient samples. We would like to thank all the patients who took part in this study and their clinicians.

Table 2. Testing for a trend in risk of CLL associated with one or more putative high-risk GST genotypes

No. of "high-risk" genotypes	GST status			Cases (n = 138)	Controls (n = 263)	Odds ratio	95% confidence interval
	GSTM1	*GSTT1*	*GSTP1*				
0	present	present	*Ile-Ile*	17	58	1	
1	present	null	*Ile-Ile*	58	100	2.0	1.1-3.7
	null	present	*Ile-Ile*				
	present	present	*Ile-Val, Val-Val*				
2	null	null	*Ile-Ile*	50	89	1.9	1.0-3.6
	null	present	*Ile-Val, Val-Val*				
	present	null	*Ile-Val, Val-Val*				
3	null	null	*Ile-Val, Val-Val*	13	16	2.8	1.1-6.9
							$P_{trend} = .04$

Downloaded from http://ashpublications.net/blood/article-pdf/99/11/4216/1685675/h8110200216.pdf by guest on 02 June 2024

References

1. Miller BA, Ries LAG, Hankey BF, Kosary CL, Harras A, Devesa SS, eds. Cancer Statistics Review 1973-90. National Cancer Institute, 1993. NIH Pub No. 93:2789-2799.
2. Yuille MR, Matutes E, Marossy A, Hilditch B, Catovsky D, Houlston RS. Familial chronic lymphocytic leukaemia: a survey and review of published studies. Br J Haematol. 2000;109:794-799.
3. Waterhouse D, Carman WJ, Schottenfeld D, Gridley G, McLean S. Cancer incidence in the rural community of Tecumseh, Michigan: a pattern of increased lymphopoietic neoplasms. Cancer. 1996;77:763-770.
4. Strange RC, Fryer AA. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. In: Vineis P, Malatus N, Lang M, et al, eds. Metabolic Polymorphisms and Susceptibility to Cancer. IARC Scientific Publications No. 148. Lyon, France: International Agency for Research on Cancer; 1999:231-249.
5. Malone KE, Koepsell TD, Daling JR, et al. Chronic lymphocytic leukemia in relation to chemical exposures. Am J Epidemiol. 1989;130:1152-1158.
6. Brown LM, Blair A, Gibson R, et al. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res. 1990;50:6585-6591.
7. Nanni O, Amadori D, Lugaresi C, et al. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on a priori exposure matrices. Occup Environ Med. 1996;53:652-657.
8. Amadori D, Nanni O, Falcini F, et al. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on job titles. Occup Environ Med. 1995;52:374-379.
9. Vinesis P, d'Errico A, Malatus N, Boffetta P. Overall evalutation and research perspectives. In: Vineis P, Malatus N, Lang M, et al, eds. Metabolic Polymorphisms and Susceptibility to Cancer. IARC Scientific Publications No. 148. Lyon, France: International Agency for Research on Cancer; 1999:403-506.
10. Lemos MC, Cabrita FJ, Silva HA, Vivan M, Placido F, Regateiro FJ. Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias. Carcinogenesis. 1999;20:1225-1229.

Downloaded from http://ashpublications.net/blood/article-pdf/99/11/4216/1685675/h8110200421 6.pdf by guest on 02 June 2024