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Genetic variations in benzene metabolism and susceptibility to multiple myeloma

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Abstract

We have previously shown that deficiency in the biotransformation enzyme glutathione-*S*-transferase theta (GSTT1) is a risk factor for multiple myeloma (MM). The present case–control study of 102 MM patients and 205 controls revealed a significant trend in increasing risk of MM with inheritance of multiple putative 'high risk' genetic variants in related pathways of benzene detoxification. Individuals who carried polymorphisms for *GSTT1* null and/or high activity microsomal epoxide hydrolase (*mEH* 113YY + 139HR or 113YY + 139RR or 113YH + 139RR) and/or low activity NAD(P)H:quinone oxidoreductase 1 (NQO1 187PS/SS) were 1.65, 2.49 and 13 times more likely to have MM ($P_{trend} = 0.001$).

Keywords: Multiple myeloma; Benzene metabolism; Polymorphisms; Genetic predisposition to disease

1. Introduction

Chronic exposure to low levels of pollutants such as those present in gasoline, cigarette smoke and automobile emissions is common in industrialised cities. However, the existence of a causal relationship between such environmental contacts and haematological malignancies, including multiple myeloma (MM), remains inconclusive and may depend on individual ability to metabolise these toxins. Such capacities are largely determined by genetic polymorphisms that affect the synthesis and/or activity of Phase I (activation) and Phase II (detoxification) enzymes involved in xenobiotic metabolism. We have previously shown that the null polymorphism for the Phase II enzyme, glutathione-*S*-transferase (*GST*)*T*1, but not *GSTM*1, is associated with a two-fold increased risk of MM [1], suggesting that small reactive hydrocarbon intermediates, detoxified by conjugation to soluble glutathione, may play a role in the aetiology of this disease.

GSTTI substrates are known to be produced by cytochrome P450 2E1 (CYP2E1) in the liver during bioactivation of organic compounds such as benzene or *N*-nitrosamine [2]. Further processing by microsomal epoxide hydrolase (mEH) or myeloperoxidase (MPO) in the bone marrow can lead to the formation of mutagenic and haematotoxic derivatives [3]. Quinone compounds produced through these pathways are reduced by NAD(P)H:quinone oxidoreductase 1 (NQO1) to protect cells from DNA damage. However, NQO1 is also responsible for the reductive activation of heterocyclic amines and dinitropyrenes, leading to formation of compounds that may promote carcinogenesis [2].

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Genetic variations that result in increased activity of the activation enzymes CYP2E1, mEH, MPO and/or decreased activity of detoxification enzymes GSTT1 and NQO1, have all been individually associated with increased susceptibility to benzene poisoning [4,5]. Chronic exposure to high levels of benzene is known to cause leukaemia [6], and even low levels of exposure can lead to hematotoxicity in susceptible individuals [7]. Conflicting evidence exists to suggest that benzene exposure could also contribute to the development of multiple myeloma [8,9]. We hypothesized that if this were the case, individual capacity to metabolise low levels of benzene encountered in the general environment could impact on the risk of disease. Thus inheritance of multiple putative 'high risk' genotypes in the benzene metabolic pathway (i.e., high activity benzene activation enzymes CYP2E1, mEH and MPO in combination with low activity detoxification enzymes GSTT1 and NQ01) could increase the risk of MM. The following case–control study was conducted to examine the frequencies of these genotypes in patients with MM compared to the general population.

2. Design and methods

2.1. Subjects

Peripheral blood or bone marrow biopsy specimens were obtained from 102 Caucasian individuals ($n = 67$ males), aged 40–91 years (median = 61 years), with confirmed diagnosis of MM according to the United Kingdom Medical Research Council (MRC UK) definition. All patients were recruited from either the Newcastle Mater Misericordiae Hospital, Newcastle, NSW or The Alfred Hospital, Melbourne, Vic., Australia. The general population frequency was calculated from 205 healthy Caucasian volunteer bone marrow donors aged 18–65 years. The research protocol was approved by the Hunter Area Research Ethics Committee and informed consent was received from all living participants.

2.2. Genotyping

DNA from each sample was genotyped for coding region single nucleotide polymorphisms (SNPs) in mEH exon 4 (H139R) and NQO1 (P187S), as well as transcription factor binding sites in the 5′ regulatory regions of MPO (−463 G/A) and CYP2E1 (−1259 *Rsa*I and −1019 *Pst*I) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using previously reported primers and restriction enzymes [10–13] (Table 1). The mEH exon 3 (Y113H) SNP was amplified using a shortened version of the previously published reverse primer [10] to avoid binding the 119 G > A substitution polymorphism known to be in tight linkage with the 113Y allele [14] and a new compatible forward primer was designed. All PCR amplifications contained 200 ng DNA, 1.5 mM $MgCl_2$, 250 μM dNTP, 0.5 μM of each primer and 1 unit of Biotaq DNA polymerase (Bioline Ltd., London, UK) in 1× Biotaq buffer as supplied by the manufacturer. All amplifications were performed in a microtube thermocycler (Corbett Research, Sydney, Australia). All oligonucleotides were synthesized by Geneworks (Adelaide, Australia) and restriction enzymes purchased from Promega (Madison WI, USA) or New England Biolabs (UK) Ltd. (Herts, UK). Products were electrophoresed through 3% Ultrapure Agarose-1000 high resolution gel (Invitrogen, Carlsbad, CA, USA) in 1× TBE buffer and visualised with ethidium bromide staining.

2.3. Statistical analysis

Genotype distributions in the general control population were tested for possible departures from the Hardy–Weinberg equilibrium by means of the Chi-square test. The frequency of genotypes in cases and controls were compared using Fisher's exact tests (two-tailed). Crude odds ratios were calculated, along with 95% confidence intervals. A test for trend (P_{trend}) in increasing risk of MM based on the number of putative risk genotypes was performed using a variance-weighted least squares test for linear trend. A $p < 0.05$ was

Table 1
Primers and enzymes used for PCR-RFLP genotyping

Gene (polymorphism)	Primers	Restriction enzyme	Banding pattern	Reference
mEH (exon 4, H139R)	tctggtgccagagcctgaccgtgc atggaacctctagcagccccgtacc	*Rsa*I	H = 300, 22 R = 177, 123, 22	[10]
NQO1 (P187S)	attctctagtgtgcctgag aatcctgcctggaagtttag	*Hin*fI	*p* = 318 S = 164, 154	[12]
MPO (−463 G/A)	cggtataggcacacaatggtgag gcaatggttcaagcgattcttc	*Aci*I	G = 169, 120, 61 A = 289, 61	[11]
CYP2E1 (−1259 *Rsa*I/−1019 *Pst*I)	ccagtcgagtctacattgtca ttcattctgtcttctaactgg	*Rsa*I/*Pst*I	c1 (*Rsa*I+/*Pst*I−) = 351, 61 c2 (*Rsa*I−/*Pst*I+) = 294, 118 c3 (*Rsa*I+/*Pst*I+) = 233, 118, 61 c4 (*Rsa*I-/*Pst*I−) = 412	[13]
mEH (exon 3, Y113H)	cttgagctctgtccttcccatccc tagtcttgaagtgacggt	*Tth111*I	T = 101 H = 83, 18	