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LETTER TO THE EDITOR

Genetic susceptibility to therapy-related leukemia after Hodgkin lymphoma or non-Hodgkin lymphoma: role of drug metabolism, apoptosis and DNA repair

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Therapy-related myelodysplasia or acute myeloid leukemia (t-MDS/AML) is a major cause of non-relapse mortality in patients treated for Hodgkin lymphoma (HL) or non-Hodgkin lymphoma (NHL).[1] t-MDS/AML is associated with exposure to alkylating agents and topoisomerase II inhibitors. The DNA-damaging events caused by these agents initiate apoptosis required for antineoplastic activity; occasionally, imperfect repair of chromosomal damage results in chromosomal aberrations, leading to leukemogenesis.

The inter-individual variability in the risk of t-MDS/AML for any given exposure to genotoxic agents suggests the role of genetic susceptibility. In addition, t-MDS/AML shares morphological and genetic similarity with *de novo* MDS/AML,[2] suggesting that therapy-related and *de novo* MDS/AML may share genetic susceptibility loci. Previous studies have been largely inconclusive, primarily because of the focus on single genes.[3,4] In the few studies where multiple genes were examined simultaneously, individuals with more than one risk variant were at higher risk.[5,6] We hypothesized that genetic variations encoded in key genes in the pathways of drug metabolism, apoptosis, DNA synthesis, methylation and repair, as well as genes involved in *de novo* AML,[7] could potentially contribute to the risk of t-MDS/AML. Using both genotype and gene expression analyses, we investigated whether individual genetic variability in these pathways modify the risk of t-MDS/AML in patients with HL or NHL exposed to genotoxic agents (Figure 1). We also tested for synergy between apoptosis and other hypothesized pathways.

Patients treated with conventional therapy or autologous hematopoietic cell transplantation (aHCT) for HL or NHL formed the sampling frame for this case-control study. Cases ($n = 49$) consisted of patients who subsequently developed t-MDS/AML. Controls ($n = 49$) were drawn from the same sampling frame, did not have t-MDS/AML and were matched to cases using the following criteria: primary diagnosis (HL or NHL), age at primary diagnosis and year of primary diagnosis, length of follow-up from diagnosis (longer for controls) and genetic ancestry. To further refine matching on genetic ancestry, we used STRUCTURE 2.0(ref. 8) to estimate ancestry composition of study subjects based on 51 informative markers (AIM)[9] (Supplementary Methods and Supplementary Table 1). Gene expression patterns were studied in hematopoietic stem/progenitor cells (HSC) from peripheral blood stem cell (PBSC) autografts from a subset of 12 NHL cases and 22 matched controls. The Human Subjects Protection Committee at City of Hope approved the protocol. Informed consent was provided according to the Declaration of Helsinki. For each study participant, cumulative therapeutic exposures were calculated, as detailed in the Supplementary Methods. Demographic and clinical characteristics of the study subjects are summarized in Supplementary Table 2.

We genotyped 29 SNPs and 2 deletion polymorphisms for 23 candidate genes (Supplementary Table 3). Association between individual polymorphisms and t-MDS/AML was analyzed using exact conditional logistic regression, adjusted for gender, treatment modality (conventional therapy vs aHCT) and cumulative exposure to alkylating agents and topoisomerase II inhibitors. We observed a higher risk for t-MDS/AML among patients with deletion of *GSTM1*, the Pro allele of P72R in *TP53*, the T allele of CYP1A1*2A, and the T allele of rs6030469 of *PTPRT* (Table 1). None of these associations withstood Bonferroni adjustment for multiple comparisons.

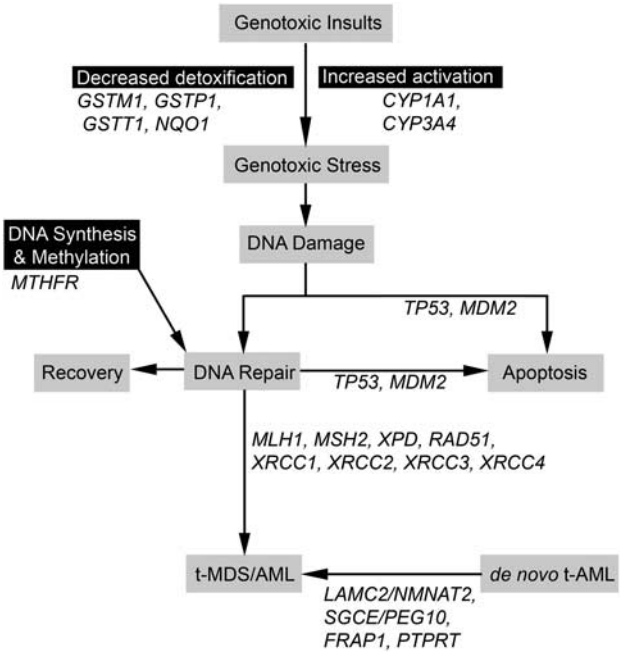

Figure 1. Candidate genes in the biological pathways implicated in the pathogenesis of t-MDS/AML.

TP53 modulates DNA repair and apoptosis upon DNA damage. A common germline polymorphism of *TP53*, P72R, produces a proline to arginine change that enhances apoptotic activity 15-fold. We used likelihood ratio tests with permutations implemented in UNPHASED[10] to explore unadjusted gene–gene interaction between P72R in *TP53* and all polymorphisms in other candidate genes. A significant interaction between P72R and C677T, a coding SNP in *MTHFR*, was detected and remained significant after correction for multiple testing using 10 000 permutations (adjusted $P_{\text{interaction}} = 0.048$). This interaction was confirmed after adjustment for therapeutic exposures (Table 1). The homozygous T allele of C677T increased the risk 71-fold ($P = 0.0059$) when combined with the Pro carrier of P72R (conferring decreased apoptotic activity) compared with its combination with homozygous Arg. We also detected an interaction between P72R and another coding SNP, A1298C, in *MTHFR* (Table 1). The homozygous A allele of A1298C increased the risk 33-fold ($P = 0.0005$) when combined with the Pro carrier of P72R compared with its combination with homozygous Arg in the adjusted analysis.

Table 1. Genetic susceptibility and risk of t-MDS–association studies and gene–gene interaction

		Case/Control	*Unadjusted*			*Adjusted*[a]		
			OR (95% CI)	P_{exact}		*OR (95% CI)*	P_{exact}	
Association studies of individual polymorphisms								
GSTM1, deletion					0.091			**0.057**
Ins/Ins		2/8	1			1		
Ins/Del		24/18	7.38 (0.92–339)	0.065		11.36 (1.03–679.9)	0.046	
Del/Del		22/23	5.98 (0.70–283)	0.14		9.47 (0.86–572.0)	0.076	
GSTM1, deletion					0.070			**0.033**
Ins/Ins		2/8	1			1		
Ins/Del or Del/Del		46/41	7.00 (0.90–315.5)	0.070		11.24 (1.05–666.0)	0.043	
*CYP1A1**2A					0.053			**0.0089**
C/C		0/3	1			1		
C/T		9/13	2.89 (0.29–∞)	0.39		1.75 (0.11–∞)	0.69	
T/T		40/33	6.77 (0.66–∞)	0.11		8.75 (0.61–∞)	0.11	
*CYP1A1**2A					0.092			**0.014**
C/C or C/T		9/16	1			1		
T/T		40/33	3.33 (0.86–18.85)	0.092		9.99 (1.25–473.5)	0.023	
TP53, P72R					0.31			**0.046**
Arg/Arg		22/30	1			1		
Pro/Arg		21/14	1.96 (0.78–5.31)	0.18		5.33 (1.16–40.71)	0.026	
Pro/Pro		6/5	1.63 (0.38–7.43)	0.65		2.35 (0.37–19.06)	0.49	
TP53, P72R					0.17			**0.039**
Arg/Arg		22/30	1			1		
Pro/Arg or Pro/Pro		27/19	1.89 (0.80–4.81)	0.17		4.05 (1.06–22.93)	0.038	
PTPRT, rs6030469					0.057			**0.030**
G/G		29/36	1			1		
G/T		9/2	3.68 (0.97–20.47)	0.057		8.92 (1.05–445.0)	0.043	
Synergistic effect of TP53 *and* MTHFR								
MTHFR C677T					0.55			1.00
T/T		7/4	1			1		
T/C or C/C		42/45	0.57 (0.12–2.25)	0.55		0.98 (0.18–5.19)	1.00	
TP53 P72R × *MTHFR* C677T					**0.0091**[b]			**0.036**[b]
P72R	C677T				**0.0060**			**0.0025**
Arg/Arg	T/T	0/4	1			1		
Arg/Arg	T/C or C/C	22/26	4.99 (0.61–∞)	0.15		7.95 (0.82–∞)	0.076	
Arg/Pro or Pro/Pro	T/C or C/C	20/19	4.99 (0.61–∞)	0.15		12.65 (1.27–∞)	0.029	
Arg/Pro or Pro/Pro	T/T	7/0	30.46 (2.44–∞)	0.0069		70.95 (3.44–∞)	0.0059	
MTHFR A1298C					1.00			0.60
A/A		24/23	1			1		
A/C or C/C		25/26	0.90 (0.32–2.46)	1.00		0.73 (0.21–2.43)	0.76	
TP53 P72R × *MTHFR* A1298C					**0.0045**[b]			**0.0035**[b]
P72R	A1298C				**0.0052**			**0.0006**
Arg/Arg	A/A	8/19	1			1		
Arg/Arg	A/C or C/C	14/11	5.40 (0.96–44.7)	0.057		8.73 (0.70–609)	0.124	
Arg/Pro or Pro/Pro	A/C or C/C	11/15	1.80 (0.34–12.1)	0.663		1.85 (0.14–33.4)	0.977	
Arg/Pro or Pro/Pro	A/A	16/4	9.11 (1.89–73.6)	0.0021		33.21 (2.91–∞)	0.0005	

Abbreviations: CI, confidence interval; OR, odds ratio; t-MDS, therapy-related myelodysplasia. [a]Adjusted for gender, aHCT (autologous hematopoietic cell transplantation; because of incomplete matching for 13 cases), alkylating agent score and topoisomerase inhibitor score. Not adjusted for multiple comparisons. Bold if <0.05 on genotype test. [b]$P_{interaction}$ between SNPs in *TP53* and *MTHFR*. Bold if <0.05.

We then examined expression levels of the 23 candidate genes using Affymetrix HG U133 plus 2.0 array data procured from an independent study that studied gene-expression profiles in CD34+ HSCs[11] to detect differential expression between cases and controls, adjusted for age at aHCT, gender and therapeutic exposures, using a general linear model implemented in R. In total, 10 genes were differentially expressed between cases and controls (Supplementary Table 4). This represents a significant enrichment ($P=0.0025$) compared with a random draw of 23 genes from a whole genome of 20 722 genes interrogated on the U133 Plus 2 array. Signals ($P<0.05$) were detected for *TP53* and genes involved in drug metabolism (*CYP3A4*, *GSTM1*, *GSTP1* and *GSTT1)*, DNA repair (*XRCC1*, *XRCC2* and *MTHFR*), and *de novo* AML (*NMNAT2* and *LAMC2*). Expression level for *TP53* was much lower in cases. Also, as hypothesized, expression of genes was observed at much lower level for the three detoxifica-

tion enzymes, *GSTM1*, *GSTP1* and *GSTT1*, whereas expression of a drug activation gene, *CYP3A4*, was significantly higher in cases. Relative expression levels measured by microarray were evaluated using TaqMan-based reverse transcribed quantitative PCR (RT-qPCR) gene-expression assays (Applied Biosystems) in 17 subjects for *GSTM1* (Hs01683722_gH), *TP53* (Hs1034249_m1) and *MTHFR* (Hs0015560_m1) referenced on *ACTB* (Hs99999903_m1); correlations were found to be statistically significant for *GSTM1* ($R^2 = 0.50$, $P = 0.0015$) and *TP53* ($R^2 = 0.24$, $P = 0.044$). Gene-expression levels of *GSTM1* and *GSTT1* were significantly correlated with deletion polymorphisms ($P < 0.0031$) in the respective genes after adjusting for multiple comparisons.

We observed associations between t-MDS/AML and genes responsible for drug metabolism supported by both genotyping and expression studies, underscoring the mechanistic relationship between t-MDS/AML and therapeutic exposures. Results were compatible with increased genotoxic stress resulting from enhanced drug activation and disrupted drug clearance among cases. Previous meta-analyses of case-control studies have demonstrated a modest increase in risk of *de novo* MDS/AML associated with the null genotypes of drug-detoxification genes, *GSTM1* and *GSTT1*, and a trend for overrepresentation for 105Val allele of *GSTP1*.[12] Thus, we confirmed an increased risk for t-MDS/AML associated with *GSTM1* homozygous deletion, and detected significantly lower gene-expression level in cases for all three genes (*GSTM1, GSTT1* and *GSTP1*). *CYP3A4* encodes a hepatic phase I P450 protein involved in activation of cyclophosphamide and some anthracycline agents. In the current study, *CYP3A4* expression in the CD34+ cells was significantly higher in cases.

We observed a higher risk for t-MDS/AML among Pro carriers of P72R in the genotyping study accompanied by a significantly lower expression level of *TP53* in cases. More importantly, we detected a synergistic effect between *TP53* and *MTHFR*. MTHFR directs 5, 10-methylene tetrahydrofolate toward methionine synthesis and conversion to the universal methyl donor, *S*-adenosylmethionine, at the expense of pyrimidine synthesis that is required for DNA synthesis and repair. Two polymorphisms, C677T and A1298C, have been associated with reduced enzyme activity.[13] Both SNPs were found to interact with P72R in increasing the risk of t-MDS/AML. *MTHFR* has been extensively investigated for susceptibility to cancer because of its key role in intracellular folate homeostasis and metabolism that are fundamental to DNA synthesis, repair and methylation. Meta-analyses has shown that the T allele of C677T polymorphism and the A allele of A1298C are associated with gastric and colorectal cancers, respectively.[14] Expression of both *TP53* and *MTHFR* was significantly lower in cases compared with controls, supporting their role in t-MDS/AML development. We proposed a model to explain the interaction between *TP53* and *MTHFR* (Supplementary Figure 1). Reduced MTHFR activity is associated with chromosomal aberrations during DNA repair.[15] When combined with higher TP53 activity, it would normally result in efficient clearance of damaged cells through apoptosis. However, when combined with less-efficient TP53, it would result in accumulation of progenitor cells with chromosomal damage and increase the risk of t-MDS/AML. On the other hand, with normal MTHFR activity to support DNA repair, allele variants of *TP53* do not have an impact on t-MDS/AML development, because efficient DNA repair would maximize DNA recovery and minimize the risk of chromosomal aberrations. The current study is the first to report a synergistic impact of SNPs in *MTHFR* and *TP53* on t-MDS/AML, however, the observation requires confirmation.

The study was limited by a relatively small sample size; the rarity of t-MDS/AML precluded a large-scale study. However, the study was strengthened by the combined use of genotyping and gene-expression analyses with detailed information regarding therapeutic exposures, allowing for the more robust (and hence clinically relevant) observations to emerge. Supporting evidence from both genotyping and expression analyses for *GSTM1*, *TP53* and *MTHFR* suggests their contribution to t-MDS/AML as germ-line genetic factors. In summary, we demonstrate that the risk of t-MDS/AML is related to the individual capacity of drug metabolism, apoptosis and DNA synthesis. These observations not only will further our understanding of the pathogenesis of t-MDS/AML but also, when confirmed in independent studies, will help identify those at the highest risk, setting the stage for targeted surveillance and/or interventions.

CONFLICT OF INTEREST

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Supplementary Information accompanies the paper on Blood Cancer Journal website (http://www.nature.com/bcj)