Brief report

A *PAI-1* (*SERPINE1*) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group

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As glucocorticoid use increased in acute lymphoblastic leukemia, osteonecrosis became an increasingly frequent complication. Besides increased age, host risk factors are poorly defined. We tested whether 12 polymorphisms were associated with osteonecrosis among patients 10 years and older treated on the CCG1882 protocol. Candidate genes (*TYMS, MTHFR, ABCB1, BGLAP, ACP5, LRP5, ESR1, PAI-1, VDR, PTH*, and *PTHR*) were chosen based on putative mechanisms underlying osteonecrosis risk. All children received dexamethasone, with doses varying by treatment arm. A *PAI-1* polymorphism (rs6092) was associated with risk of osteonecrosis in univariate (*P* = .002; odds ratio = 2.79) and multivariate (*P* = .002; odds ratio = 2.89) analyses (adjusting for gender, age, and treatment arm). Overall, 21 of 78 (26.9%) children with *PAI-1* GA/AA genotypes, versus 25 of 214 (11.7%) children with GG genotype, developed osteonecrosis. *PAI-1* polymorphisms and PAI-1 serum levels have previously been associated with thrombosis. We conclude that *PAI-1* genetic variation may contribute to risk of osteonecrosis. (Blood. 2008;111: 4496-4499)

Introduction

As cure rates for childhood acute lymphoblastic leukemia (ALL) increased, adverse effects of chemotherapy became an important area of study. Osteonecrosis is one of these, attributed primarily to corticosteroids (dexamethasone and prednisone) that play a vital role in treatment of ALL, as they have improved cure rates.[1] Osteonecrosis may lead to severe joint pain, limitations on physical activity, with some cases culminating in surgical intervention to restore function.[2] Osteonecrosis affects up to one-third of patients treated for ALL, and host-related factors such as age more than 10 years, female sex, and white race increase risk of development.[3-5] Better definition of risk factors for osteonecrosis might permit tailoring therapy to minimize this complication.

We have shown that therapy-related adverse events (gastrointestinal, infectious, hepatic, and neurologic toxicities) can be attributed to germline polymorphisms in genes linked to pharmacodynamics of chemotherapy.[6] In a relatively small series of patients at high risk of osteonecrosis, we found 2 inherited genetic polymorphisms, vitamin D receptor FokI start site polymorphism and thymidylate synthetase enhancer repeat, were associated with risk of osteonecrosis.[5] To further explore genetic predictors of osteonecrosis, we herein studied 12 candidate polymorphisms potentially involved in osteonecrosis in a mature, completed study of the Children's Cancer Group (CCG1882), whose protocol resulted in a relatively high incidence of osteonecrosis in children 10 years and older.[4]

Methods

Approval was obtained from St Jude Children's Research Hospital institutional review board for these studies (institutional review board approval number XMP03-045 for protocol AALL03B2, Study of pharmacogenetic risk factors for avascular necrosis approved by the Children's Oncology Group). Informed consent was obtained in accordance with the Declaration of Helsinki.

A total of 980 patients were 10 years of age or older when enrolled on CCG1882; 108 developed symptomatic osteonecrosis (11.0%) diagnosed by radiographic imaging; patients were not prospectively screened with radiologic imaging.[4] A total of 361 patients had sufficient archived DNA for inclusion in genotyping; 51 of these patients developed symptomatic osteonecrosis, an incidence not significantly different from the overall CCG1882 population (*P* = .188; Table 1). Patients were scheduled to receive the following total doses of steroids: during induction, prednisone (1815 mg/m^2); delayed intensification, dexamethasone (standard arm, 235 mg/m^2; augmented arm, 470 mg/m^2); maintenance, prednisone (standard arm: males = 7000 mg/m^2, females = 4400 mg/m^2; augmented arm: males = 6200 mg/m^2, females = 3600 mg/m^2).[4]

DNA was isolated from archived diagnostic tissue samples preserved on glass slides, including bone marrow aspirates and peripheral blood smears.[7]

Genotyping was performed for 12 candidate polymorphisms (Table 2): *TYMS* enhancer repeat, *VDR* start site Fok1 (rs2228570[8]),[5] *MTHFR* C677T (rs1801133), *PAI-1* (rs6092),[9] *ESR* PvuII (rs2234693), *LRP5* (rs2306862), *BGLAP* (rs1800247), *ACP5* (rs2305799 and rs2229531, respectively), *ABCB1* (rs1045642), *PTH* (rs6254), and *PTHR* (rs1138518). The *TYMS*

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Table 1. Demographics and osteonecrosis status of the study population

	Developed osteonecrosis?		*P*	
	Yes	No	Original entire CCG1882 cohort	Subset included herein
Age			.290	.569
10 to 15 years	43	248		
16 to 20 years	8	62		
Sex			.030	.034
Male	21	178		
Female	30	132		
Race			.003	.035
White	45	212		
Black	2	28		
Hispanic	4	55		
Other	0	15		
Treatment arm RER			.780	1.000
XRT	24	163		
No XRT	7	51		
Treatment arm SER			.270	.459
Standard	7	45		
Augmented	13	51		

RER indicates rapid early response; SER, slow early response; and XRT, cranial irradiation therapy.

enhancer repeat was tested as described.[5] With samples that failed initial testing, a second set of primers was designed to produce smaller 116 and 144 bp products (corresponding to an enhancer 2-repeat or 3-repeat variation).[10] For all polymorphisms except *TYMS*, genotyping was performed using the Beckman Coulter GenomeLab SNPstream (Fullerton, CA) by DNAprint. Additional genotyping for the *VDR* start site Fok1 polymorphism for samples failing initial genotyping was performed as described.[7] Not all patients were successfully genotyped for each polymorphism (Table 2; www.pharmgkb.org PS207425).

Univariate analysis was performed using logistic regression for each individual genotype as a predictor for osteonecrosis. Multivariate logistic regression analyses were performed for genotype, adjusting for age (10-15 years and 16+ years), sex, and treatment (rapid early response patients: with vs without prophylactic cranial radiation; slow early response patients: standard vs augmented; Table 2). Adjustment for ethnicity was not performed because of a low number of patients of nonwhite ethnicity (n = 6) affected by osteonecrosis (Table 1). Logistic regression analyses were performed to test for possible correlations among polymorphisms. Among the largest racial/ethnic group (whites), we compared whether genotype distributions varied from those expected under Hardy-Weinberg assumptions using a χ^2 test; only the *TYMS* and *LRP5* polymorphisms varied from Hardy-Weinberg predictions ($P < .004$).

Results and discussion

A total of 43 of 291 patients who were 10 to 15 years of age and 8 of 70 patients 16 years of age and older developed osteonecrosis (Table 1). Osteonecrosis was more common in females and whites ($P = .034$ and $P = .035$, respectively; Fisher exact test). Univariate analysis (Table 2) indicated that only the *PAI-1* polymorphism was associated with osteonecrosis ($P = .002$), with an odds ratio of 2.79 (95% confidence interval, 1.45-5.34). A total of 26.9% of the combined GA and AA genotypes developed osteonecrosis compared with 11.7% of the GG genotypic group.

Multivariate logistic regression analysis adjusted for age, sex, and treatment arm (Table 2) did not significantly alter the results: *PAI-1* was associated with osteonecrosis ($P = .002$ and adjusted odds ratio = 2.89). Logistic regression analysis revealed no significant correlations among genotypes.

PAI-1 rs6092 allele frequencies differed significantly between racial/ethnic groups. The combined GA and AA genotypes were present in 30.9% of whites but only 12.5% of blacks. The incidence of GA or AA genotype in Hispanics was 20%, not significantly different from the white population ($P = .572$).

Other than older age,[4,5,11-14] host-related risk factors for osteonecrosis remain incompletely defined. Herein, after adjusting for age, sex, and treatment arm, the only genetic risk factor for osteonecrosis was a *PAI-1* polymorphism.

PAI-1 inhibits fibrinolysis. Increased serum levels have been associated with increased incidence of thrombophilia[15] and osteonecrosis,[16-19] although reports are not consistent. High levels of PAI-1, induced by corticosteroid treatment,[20] or through polymorphisms in *PAI-1*, lead to suppression of fibrinolysis through inhibition of tissue plasminogen activator and promotion of thrombosis.[21] Resulting increased intraosseous venous pressure blocking blood flow to the femoral head may culminate in hypoxic bone death or osteonecrosis.[17,19]

The *PAI-1* SNP (rs6092) we found associated with osteonecrosis resides in a haplotype containing a 4G/5G repeat promoter insertion/deletion polymorphism.[9] The 4G allele has been linked with variation in PAI-1 serum levels,[9,22,23] metabolic syndrome, coronary atherosclerosis, myocardial infarction, increased serum triglycerides,[24] and also with increased risk of osteonecrosis among renal transplant recipients who received glucocorticoids.[25] Our findings confirm an association of *PAI-1* germ line variation with osteonecrosis risk in an entirely different clinical setting: children who received glucocorticoids as part of ALL chemotherapy.

We did not confirm our prior finding[5] in a St Jude ALL cohort that polymorphisms in *VDR* and *TYMS* were associated with osteonecrosis. This discrepancy may be related to technical challenges posed by DNA quality or to differences in chemotherapy between the St Jude and CCG cohorts, in that patients at St Jude received more antimetabolites (particularly methotrexate) than patients on CCG1882. Methotrexate is associated with high plasma homocysteine and folate depletion, both of which have been linked to thrombosis and osteonecrosis.[26,27] Thus, it is plausible that lower expression of thymidylate synthetase (a target of methotrexate) associated with the *TYMS* polymorphism may be more relevant for patients receiving methotrexate-intensive chemotherapy regimens but perhaps not for patients receiving other ALL regimens. As in all of pharmacogenetics, the important target genes will depend on therapy.

Interestingly, the risk of osteonecrosis was higher among whites than other ethnic/racial groups in this study, as well as in the larger CCG1882 cohort and in another group ALL cohort, with incidence in the larger CCG1882 cohort being approximately 2.5-fold more common in whites than nonwhites.[4,5] Although the number of nonwhites in our cohort available for genotyping was relatively small and therefore limits our power, the magnitude of increased osteonecrosis risk associated with the *PAI-1* A allele (~2.8-fold) is similar to the increased frequency of harboring at least one A allele in whites compared with nonwhites (~2.4-fold), suggesting that racial differences in germline genomic variations might account for differences in frequency of osteonecrosis among racial/ethnic groups.

Table 2. Univariate and multivariate analyses of genotype versus development of osteonecrosis

Gene, rs no/genotype	Patients successfully genotyped, %	Osteonecrosis, no.		Univariate analysis			Multivariate analysis		
		Yes	No	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
Thymidylate synthetase (*TYMS*) enhancer repeat	86			1.40	0.72-2.74	.326	1.42	0.72-2.80	.319
2R/2R		16	80						
All others		27	189						
Vitamin D receptor start codon Fok1 (*VDR*) rs2228570	84			1.70	0.82-3.53	.153	1.91	0.90-4.03	.091
TT/CT		32	164						
CC		11	96						
Osteocalcin (*BGLAP*) rs1800247	66			0.87	0.28-2.73	.817	0.88	0.27-2.84	.824
TT/CT		32	183						
CC		4	20						
Estrogen receptor alpha (*ESR1*) rs2234693	61			0.68	0.32-1.46	.328	0.64	0.29-1.42	.276
CC/CT		13	95						
TT		19	95						
Low-density lipoprotein receptor-related protein (*LRP5*) rs2306862	70			0.72	0.36-1.41	.336	0.68	0.34-1.37	.281
TT/CT		18	113						
CC		22	99						
5,10-Methylenetetrahydrofolate reductase (*MTHFR*) rs1801133	71			1.30	0.68-2.51	.432	1.22	0.62-2.41	.563
TT/CT		22	95						
CC		21	118						
Plasminogen activator inhibitor-1 (*PAI-1*) rs6092	81			2.79	1.45-5.34	.002	2.89	1.48-5.62	.002
AA/GA		21	57						
GG		25	189						
P-glycoprotein (*ABCB1*) rs1045642	77			1.35	0.65-2.82	.422	1.52	0.71-3.23	.278
TT/CT		33	162						
CC		11	73						
Parathyroid hormone (*PTH*) rs6254	76			0.75	0.39-1.44	.393	0.75	0.38-1.47	.404
AA/GT		19	115						
GG		25	114						
Parathyroid hormone receptor (*PTHR*) rs1138518	69			0.69	0.34-1.42	.313	0.72	0.34-1.50	.377
CC/TC		16	117						
TT		19	96						
Tartrate-resistant acid phosphatase (*ACP5*) rs2229531	73			0.81	0.30-2.22	.683	0.84	0.30-2.36	.733
AA/GT		5	31						
GG		38	191						
Tartrate-resistant acid phosphatase (*ACP5*) rs2305799	77			0.77	0.34-1.75	.527	.82	0.35-1.92	.650
TT/CT		8	54						
CC		35	181						

CI indicates confidence interval.

Our findings support the hypothesis that inherited variation in *PAI-1* contributes to variation in risk of constitutive and drug-induced phenotypes and to the notion that some individuals are more prone to develop serious adverse effects of ALL therapy than others.

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Authorship

Contribution: L.A.M., J.B.N., and M.V.R. conceived and designed the project; H.N.S. and M.D. carried out and interpreted the statistical analyses; D.F. and L.H.H. interpreted the data and were primary authors; all authors contributed to the writing of the paper.

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