[haematologica]
2004;89:664-670

Polymorphisms of CYP1A1 and glutathione S-transferase and susceptibility to adult acute myeloid leukemia

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A B S T R A C T

Background and Objectives. The origin of acute myeloid leukemia (AML) may be explained by a combination of genetic susceptibility factors and environmental exposure. We studied the polymorphisms of cytochrome P450 CYP1A1 and glutathione S-transferase (GST), enzymes involved in the metabolism of carcinogens and anti-cancer drugs, as risk factors for adult AML.

Design and Methods. The prevalence of CYP1A1*2A, *2B and *4 alleles and of GSTM1 and GSTT1 homozygous deletions was examined in 193 patients with AML and 273 normal individuals using polymerase chain reaction (PCR)-based methods.

Results. A higher prevalence of the CYP1A1*4 allele was found in AML patients than in controls (19.1% vs 9.9%, OR =2.2, 95% C.I. 1.3-3.7, *p*=0.006). GSTT1 homozygous deletions were also more frequent in AML patients (29% vs 19%, OR = 1.7, 95% CI 1.1-2.7, *p*=0.02). The combination of GSTT1 null genotype and CYP1A1 *2B and *4 alleles further increased the risk of AML (OR =10.2, 95% CI 1.2-83.9, *p*=0.01, and OR =7.0, 95% CI 2.0-24.8, *p*=0.001, respectively).

Interpretation and Conclusions. Polymorphic variants in xenobiotic-metabolism genes, including CYP1A1 and GSTT1, may increase the risk of adult AML, particularly when present togheter.

Key words: glutathione S-transferase, CYP1A1, acute myeloid leukemia.

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DNA damage in the hematopoietic precursor cell is the essential prerequisite for the development of acute myeloid leukemia (AML). Such damage may result from the interaction of reactive species generated by environmental or endogenous metabolites. Humans vary in their ability to metabolize such reactive intermediates, which may explain differences in leukemia risk as a result of the interplay of genetic susceptibility and exogenous exposure. The carcinogenic effect of xenobiotics is influenced by a series of genes codifying enzymes involved in oxidation/activation (phase I) and conjugation/detoxification (phase II) of these compounds. Polymorphisms of these genes, resulting in functional allelic variants of the corresponding enzymes, have been shown to influence the risk of developing solid tumors and hematologic malignancies and can also modify individual response to cytotoxic treatment.[1-10] We have previously shown that homozygous deletions of two detoxification enzymes, the glutathione S-transferase (GST) M1 and/or GSTT1, predict a negative prognosis in adults with AML.[11]

GST enzymes detoxify environmental carcinogens, such as benzo(a)pyrene and other polyaromatic hydrocarbons, but also anticancer drugs, including alkylating agents, anthracyclines and cyclophosphamide metabolites. Homozygous deletions of GSTM1 and GSTT1 are present in a large proportion of individuals as a genetic polymorphism. This causes absence of the specific enzymatic activity. The risk for some solid tumors is particularly increased when GST homozygous deletions are associated with polymorphisms of enzymes involved in phase I reactions and prolonged exposure to carcinogens, such as tobacco.[3-6]

CYP1A1 belongs to the cytochrome P450 family and is a phase I enzyme involved in the bioactivation of several chemical carcinogens, including polycyclic aromatic hydrocarbons (PAH).[9] Oxidation of PAH produces an epoxide, a very reactive electrophilic group which can interact with DNA resulting in the formation of DNA adducts. Usually these epoxides are rapidly hydrolyzed into hydroxyl groups, which are then coupled to glucuronic acid, glutathione or other groups, producing water-soluble compounds that can then be excreted (phase II).[9,12] Polymorphisms in the CYP1A1 gene have been described. The T6235C mutation is located 1194 bp downstream of exon 7 and when present alone corresponds to the CYP1A*2A allele.[13] The A4889G mutation results in replacement of Ile by Val at residue 462 in exon 7, corresponding to the heme-binding region of CYP1A1. This mutation is in linkage disequilibrium with the T6235C mutation (CYP1A1*2B allele).[14] The C4887A mutation results in the replacement of Thr by Asn in codon 461, near the site of the A4889G mutation (CYP1A1*4 allele).[15] The CYP1A1 variants *2A and *2B have an increased enzymatic activity and/or inducibility, while the biological significance of the *4 allele is still unknown.[15]

We studied the frequency of CYP1A1 allelic variants and GSTM1 and T1 homozygous deletions in a group of Italian AML patients and compared frequencies to those in a control group of similar age and sex distribution with a negative history for neoplastic diseases. Allelic variants were then correlated to clinical and biological characteristics of the patients.

Design and Methods

Patients' characteristics

Our retrospective analysis included 193 patients (86 females, 107 males, median age 62 years, range 19-87 years), consecutively diagnosed with AML between February 1992 and October 2003. Bone marrow or peripheral blood samples were obtained at the time of initial diagnosis. The diagnosis of AML was defined according to morphology and phenotype, following the WHO classification criteria, and a blast percentage cut-off of 20% was used.[16] The presence of myelodysplasia was determined by two different experts by inspection of 300 marrow nucleated cells and 20 megakaryocytes on bone marrow smears stained with May-Grünwald-Giemsa panoptical stain. The diagnosis of AML *with myelodysplasia* was established when myelodysplatic features involved two or more cell lineages and were present in more than 10% of the marrow cells.[17] Thirty-four patients had leukemia following another malignancy. Seventeen patients had been treated with chemotherapy for the primary tumor and 8 with radiotherapy and were defined as having therapy-related leukemias. Nine patients had been managed only by surgery. Karyotype, obtained at the time of initial diagnosis, was available for 132 patients. White blood cell (WBC) count and blast percentage data were available for 145 and 137 patients, respectively. The median WBC count was 6.9×10^9/L (range 0.3-380×10^9/L), while the median bone marrow blast percentage was 58% (range 20-100%). Peripheral blood samples from 273 normal individuals (126 females, 147 males, median age 60 years, range 19-90 years) were used as control samples. These individuals had no medical history of any type of cancer and were not related to the patients. All patients and controls were Italians, from central and southern regions of the country. Informed consent was obtained from all patients and controls, according to institutional guidelines.

DNA extraction and amplification

Mononuclear cells (MNC) were separated from the bone marrow of AML patients at the time of initial diagnosis, using Ficoll density centrifugation at 400 g for 20 minutes. Leukocytes were freshly isolated from peripheral blood of controls following hypotonic lysis of the red cells. Cells from AML patients and controls were then washed twice with PBS at 400 g and 4°C for 7 minutes, and DNA was extracted using DNAzol (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. CYP1A1 mutations T6235C, A4889G and C4887A were characterized by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, as described by Cascorbi *et al.*[15]

Homozygous deletions of GSTM1 and GSTT1 were studied using a multiplex PCR technique, including primers for the housekeeping gene BCL-2 as the internal control, as previously described.[11]

Statistical analysis

The statistical significance of the differences between groups was calculated using Fisher's exact test (two-sided). Absolute odds ratios (OR) were calculated separately for CYP1A1*2A, *2B and *4 and for GSTM1 and GSTT1 null genotypes and are given with 95% confidence intervals (CI). Age, gender, morphology, history of treatment for previous cancer, cytogenetics, blast percentage and WBC count were included as co-variables. Cytogenetic risk groups were defined according to Grimwade *et al.*[18] as favorable: t(8;21), t(15;17), inv(16); intermediate: normal, +8, +21, +22, del(7q), del(9q), abnormal 11q23, all other structural/numerical abnormalities without additional favorable or adverse cytogenetic changes; and unfavorable: -5, -7, del(5q), abnormal 3q, complex karyotype. All computations were performed using Stata 6.0 software (Stata Corp., College Station, TX, USA).

Results

Prevalence of CYP1A1 alleles and GST homozygous deletions

Our study included 193 patients with adult AML and 273 normal controls. The observed frequency of CYP1A1 alleles and GST homozygous deletions in the control population was similar to that reported by other authors for Caucasians (Table 1).[7,19-22] No significant differences in the prevalence of the *2A and *2B alleles were found when comparing AML patients and controls (Table 1). A higher prevalence of CYP1A1*4 allele was found in AML patients (p=0.006) and was associated with a 2.2-fold increased risk of acute myeloid leukemia (95% CI 1.3 -3.7)(Table 1). Moreover when analyzing the frequency of the CYP1A1*4 allele in patients and controls, grouped according to sex and age, we found a higher prevalence of this allele in female patients than in female controls (18.6% vs 7.1%, p=0.02, OR 3.0, 95% CI 1.2-7.1), in particular in females younger than 60 years (24.4% vs 7.1%, p=0.02, OR 4.2, 95% CI 1.2-14.5) (Table 2).

A similar frequency of GSTM1 homozygous deletions was found in AML patients and controls (42.5% vs 46.9%). GSTT1 deletions were more frequent in patients with AML (29% vs 19%), and there was a significantly increased risk of AML associated with this genotype (OR= 1.7; 95% CI 1.1.-2.7, p=0.02) (Table 1). The double null genotype was detected in 13.5% of patients and 9% of controls. We next examined whether the associations between CYP1A1 polymorphisms and GST homozygous deletions conferred an additional AML risk. No significant association between CYP1A1*2A, *2B and *4 variant alleles and GSTM1 homozygous deletions were found (*data not shown*). A significantly increased AML risk was evident for patients with the combined GSTT1 null genotype and the CYP1A1 *2B allele (OR=10.2, p=0.01) or GSTT1 null genotype and CYP1A1 *4 allele (OR=7.0, p=0.001) (Table 3). The combination of GSTT1 homozygous deletions with CYP1A1 alleles *4 and/or *2B conferred a 7.8-fold increased risk of AML (p<0.0001; 95% CI 2.6-23.1) .

CYP1A1 and GST genotypes and patients' characteristics

We next concentrated our analysis on the group of AML patients. For this purpose, we examined the CYP1A1 and GST genotypes by grouping patients according to age, gender, history of previous cancer, morphology, cytogenetics, WBC count and blast percentage at the time of the diagnosis (Table 4). No association was found between gender and GST null genotypes, but there was a higher frequency of GSTT1 homozygous deletions and GSTT1/GSTM1 double null genotypes in patients over 60 years of age (OR=1.97, 95% CI 1.01-3.8, p=0.05 and OR =2.7, 95% CI 1.02 –7.0, p= 0.05, respectively), confirming our previous results from a smaller number of patients.[11] The GSTT1 null genotype was an independent risk factor for AML in the elderly (p=0.04), also in a multivariate analysis including age, CYP1A1*4 allele, GSTM1 null and GSTT1 null genotypes.

Table 1. Distribution of CYP1A1 and GST genotypes in AML patients and controls.

Polymorphism	*Genotype**	*Cases N./total (%)*	*Controls N./total (%)*	*p°*	*Odds Ratio (95% C.I.)*
CYP1A1 *2A (#)	-/+	17/178 (9.6)	42/268 (15.7)		
	+/+	0/178 (0)	0/268 (0)	0.06	
CYP1A1 *2B	-/+	14/193 (7.3)	25/273 (9.2)		
	+/+	0/193 (0)	0/273 (0)	0.5	
CYP1A1 *4	-/+	35/193 (18.1)	26/273 (9.5)	**0.006**	2.2 (1.3 –3.7)
	+/+	2/193 (1.0)	1/273 (0.4)		
GSTM1	del/del	82/193 (42.5)	128/273 (46.9)	0.4	
GSTT1	del/del	56/193 (29.0)	52/273 (19)	**0.02**	1.7 (1.1-2.7)

**-/+ and +/+ are respectively heterozygous and homozygous mutants for a given allele; °heterozygous and homozygous mutant genotypes are considered together in the estimates of p and the odds ratio; #CYP1A1 *2A allele was successfully genotyped only in 178 AML patients and 268 controls. Statistically significant data are shown in bold.*

Table 2. Distribution of CYP1A1 *4 allele according to sex and age.

Age		*Female*	*Male*
All ages	AML patients (%)	16/86 (18.6)	21/107 (19.6)
	Controls (%)	9/126 (7.1)	17/147 (11.6)
	p	**0.02**	0.1
	O.R. (95% C.I.)	**3.0 (1.2-7.1)**	–
≤ 60 years	AML patients (%)	10/41 (24.4)	9/42 (21.4)
	Controls (%)	4/56 (7.1)	9/86 (10.5)
	p	**0.02**	0.1
	O.R. (95% C.I.)	**4.2 (1.2-14.5)**	–
> 60 years	AML patients (%)	6/45 (13.3)	12/65 (18.5)
	Controls (%)	5/70 (7.1)	8/61 (13.1)
	p	0.3	0.5
	O.R. (95% C.I.)	–	–

Statistically significant data are shown in bold.

Table 3. GSTT1 null genotype combined with CYP1A1 polymorphisms and AML risk.

GSTT1	*CYP1A1 *2B*	*AML patients (n=193)*	*Controls (n=273)*	*OR*	*95% C.I.*	*p*
+/+; +/-	-/-	132	197	0.8	0.6-1.2	0.4
+/+; +/-	-/+; +/+	7	24	**0.4**	**0.2-0.9**	**0.04**
-/-	-/-	47	51	1.4	0.9-2.2	0.17
-/-	-/+; +/+	7	1	**10.2**	**1.2-83.9**	**0.01**
GSTT1	*CYP1A1*4*					
+/+; +/-	-/-	116	197	**0.6**	**0.4-0.9**	**0.007**
+/+; +/-	-/+; +/+	23	24	1.4	0.8-2.6	0.27
-/-	-/-	40	49	1.2	0.7-1.9	0.47
-/-	-/+; +/+	14	3	**7.0**	**2.0-24.8**	**0.001**

*GSTT1 -/- indicates the presence of homozygous deletions, while +/+ and +/- signify that at least one GSTT1 allele is carried. CYP1A1 *2B and *4 -/+ and +/+ indicate carriers of these variants. Statistically significant data are shown in bold.*

As GST and CYP1A1 are involved in detoxification of both natural and drug carcinogens,[1,5,23,24] we analyzed the distribution of enzymatic polymorphisms in patients who had received chemo- and/or radiotherapy for a previous cancer. No differences were observed for GST and CYP1A1 variants when comparing *de novo* versus therapy-related AML (Table 4). We then grouped patients according to morphology, by splitting them in two groups, those with and those without myelodysplasia.[16,17] The CYP1A1*2B allele was more frequent in AML patients with multilineage dysplasia: it was present in 5 of 30 AML patients with myelodysplasia, corresponding to 16.7%, as compared to other AML subtypes in which the 2B allele was present in 7 of 145 patients, corresponding to 4.8% (OR= 3.9, 95% CI 1.2-13.4, p=0.03). The independent association of CYP1A1*2B and presence of myelodysplasia was confirmed by the multivariate analysis (p=0.01), also including cytogenetic risk groups as a co-variable. On the other hand, no differences were found in the prevalence of GSTM1 and T1 homozygous deletions and of CYP1A1*2A and *4 alleles between the two groups of patients.

Cytogenetic data were available for 132 patients. Fifty-seven patients had a normal karyotype, 36 had a simple balanced translocation and 39 patients had a complex karyotype. This corresponds to 32 patients with prognostically favorable cytogenetics, 75 with intermediate and 25 with unfavorable cytogenetics, according to Grimwade *et al.*[18] No differences were found in the frequency of CYP1A1 alleles and GST homozygous deletions when comparing the different groups (Table 4).

No associations were found between GST null genotypes and CYP1A1 polymorphisms and WBC count at diagnosis (Table 4). In contrast, we found a significant association between the CYP1A1 *4 allele and a blast percentage lower than 50% (p=0.02)[25] (Table 4).

Discussion

In this study, we report that frequencies of some polymorphisms in xenobiotic metabolizing enzymes, the GSTT1 null genotype and the cytochrome P450 CYP1A1*4 allele were significantly higher in patients with AML than in controls. The GSTT1 deletion conferred a 1.7-fold increase in the risk of AML and the CYP1A1*4 allele a 2.2-fold increase. The risk was even greater when homozygous deletion of GSTT1 was combined with CYP1A1*4, the OR of this combination being 7.0. Although the CYP1A1*2B allele by itself was not associated with an increased risk of AML, an interaction with the GSTT1 null genotype became evident, as the combination of the two genotypes conferred a higher risk than the GSTT1 null genotype alone (OR 10.2). Moreover, the combination of GSTT1 null genotype with either alleles of CYP1A1, resulting in an amino acid change in the heme-binding region of the enzyme, was associated with a 7.7-fold increased risk of AML. As the equilibrium between activating (phase I) and detoxifying (phase II) enzyme activities is critical in the host's response to xenobiotics, our data suggest a possible accumulation of reactive intermediates in individuals with higher CYP1A1 activity and deletion of GST, increasing the risk of DNA damage and contributing to leukemogenesis. To our knowledge, this is the first study assessing the risk of AML in individuals with polymorphic changes in both phase I and phase II enzyme genes. Similarly, the association of CYP1A1*2A and *2B alleles with GSTM1 null genotype was shown to increase the risk of cigarette-relat-

Table 4. Patients' characteristics and GST and CYP1A1 polymorphisms.

		CYP1A1 alleles			GST genotypes	
	(tot)	*2A (°) n/tot (%)	*2B n (%)	*4 n (%)	M1 null n (%)	T1 null n (%)
Age (years)	≤ 60 (83)	5/76 (6.6)	4 (4.8)	19 (22.9)	30 (36.1)	17 (20.5)
	> 60 (110)	12/102 (11.8)	10 (9.1)	18 (16.4)	52 (47.3)	37 (33.6)
	p	0.3	0.4	0.2	0.1	**0.05**
Sex	Female (86)	7/79 (8.9)	7 (8.1)	16 (18.6)	36 (41.9)	25 (29.1)
	Male (107)	10/99 (10.1)	7 (6.5)	21 (19.6)	46 (42.9)	29 (27.1)
	p	0.8	0.8	0.9	0.9	0.9
Previous cancer	*De novo* (168)	17/158 (10.7)	11 (6.5)	32 (19)	73 (43.5)	50 (29.8)
	Therapy-related (25)	0/20 (0)	3 (12)	5 (20)	9 (36)	4 (16)
	p	0.2	0.4	0.8	0.5	0.2
Karyotype (n=132)	Normal (57)	5/50 (10)	4 (7)	11 (19.3)	21 (36.8)	17 (29.8)
	°S. Translocation (36)	2/32 (6.3)	1 (2.8)	6 (16.7)	18 (50)	11 (30.6)
	Complex (39)	5/36 (13.9)	1 (2.6)	6 (15.4)	15 (38.5)	9 (23.1)
	p	0.6	0.6	0.9	0.4	0.7
Cytogenetic risk group (n=132)	Favorable (32)	3/30 (10)	0 (0)	5 (15.6)	17 (53.1)	10 (31.3)
	Intermediate (75)	7/66 (10.6)	6 (8)	16 (21.3)	27 (36)	23 (30.7)
	Unfavorable (25)	2/22 (9.1)	0 (0)	2 (8)	10 (40)	4 (16)
	p	1.0	0.1	0.6	0.3	0.4
Presence of myelodysplasia (n=175)	Yes (30)	2/27 (7.4)	5 (16.7)	5 (16.7)	10 (33.3)	11 (36.7)
	No (145)	14/139(10.1)	7 (4.8)	25 (17.2)	63 (43.4)	38 (26.2)
	p	1.0	**0.03**	1.0	0.4	0.3
WBC count (n=145)	≥ 30×10^9/L (47)	4/43 (9.3)	4 (8.5)	11 (23.4)	20 (42.6)	13 (27.7)
	< 30×10^9/L (98)	9/91 (9.9)	5 (5.1)	20 (20.4)	38 (38.7)	28 (28.6)
	p	1.0	0.5	0.7	0.7	1.0
Blast percentage (n=137)	≥ 50% (81)	8/74 (10.8)	4 (4.9)	11 (13.5)	30 (37)	20 (24.6)
	< 50% (56)	3/53 (5.7)	4 (7.1)	17 (30.3)	27 (48.2)	19 (33.9)
	p	0.7	0.7	**0.02**	0.5	0.6

*Statistically significant data are shown in bold. (°) CYP1A1 *2A allele was successfully genotyped only in 178 AML patients and 268 controls.*

ed lung carcinoma,[26] and the combination of CYP1A1*2A and GSTM1 null was found to be a significant predictor of the risk of childhood acute lymphoblastic leukemia.[7,21]

The only previous report on the prevalence of CYP1A1 polymorphisms in adult leukemia showed no differences in the distribution of CYP1A1 genotypes between AML patients and controls, but in that case only the frequencies of the *2A and *2B alleles were studied.[22] Here, we show an increased risk of AML associated with the *4 alleles of CYP1A1, particularly in female AML patients under 60 years of age. The functional significance of the CYP1A1*4 variant is still unknown but it might influence activation of polycyclic aromatic hydrocarbons (PAH) and estradiol metabolism. It has been suggested that electrophilic quinones, derived from the oxidation of PAH such as benzene and from endogenous and exogenous estrogens, may react with DNA, particularly at N-7 of guanine and N-3 of adenine, to form adducts which are finally released by destabilization of the glycosyl bond generating apurinic sites. This has been considered a critical event leading to oncogenic mutations and the initiation of cancer.[27] Evidence supporting this hypothesis has been obtained from human breast and animal models susceptible to estrogen-induced tumors.[27] Similarly, the CYP1A1*4 allele was found to be a significant risk determinant for breast carcinoma, particularly among post-menopausal women,[8] and for endometrial carcinoma.[27]

In our study, we found a higher incidence of CYP1A1 *2B allele in patients with AML associated with multilineage dysplasia with or without a previous myelodysplastic syndrome[16] than in the other types of

AML (16.7% vs 4.8%, p=0.03). Bowen *et al.* reported that the CYP1A1*2B variant allele was over-represented in patients with AML who were carriers of N-RAS mutation and had a poor-risk karyotype, comprising partial/complete deletion of chromosome 5 or 7, or abnormalities of chromosome 3.[29] N-Ras mutations have been shown to be a crucial step in 7,12-dimethylbenz[a]anthracene (DMBA)-induced leukemogenesis in an animal model,[30] and they are described in 15-30% of AML and MDS.[31] The higher enzymatic activity and inducibility of CYP1A1 *2B allele can increase the activation of environmental carcinogens, leading to formation of DNA adducts and N-ras mutations and may be implicated in the pathogenesis of myelodysplastic changes. On the other hand, we did not find any differences in the frequency of CYP1A1 variants and GST deletions in therapy-related AML, in contrast to previous reports.[32,33] Probably other enzyme polymorphisms are involved in determining the risk of secondary leukemia, as shown for glutathione S-transferase P1 (GSTP1), NAD(P)H:quinone oxidoreductase (NQOI) and cytochrome CYP3A4.[34-36]

Previous reports on GST null genotypes as a risk factor for AML and MDS gave heterogeneous results. A study from Britain reported an association between GSTT1 null and GSTM1 null genotypes and risk of AML (OR 1.32 and 1.24, respectively),[37] a study from the United States found an increased risk for AML in children with the GSTM1 null genotype,[38] and an epidemiological study from Japan showed that individuals with GSTT1 null genotype are at a 2.4-fold higher risk of developing therapy-related AML and AML with trilineage dysplasia.[39] Other studies failed to confirm this association.[23,40,41]

The reasons for these discrepancies are unclear. Given the association between GST null genotypes and solid tumors, it is important that the control group has no previous history of malignancies, and have a racial and geographic origin similar to that of patients. In our study, the prevalence of GSTM1 and GSTT1 null genotypes and of CYP1A1 alleles in the published range for Caucasian populations.[7,19-22] This suggests that the differences in genotype frequencies between patients and controls were not related to a sampling problem in the control group.

The discrepancies between published results on the association of leukemia risk and gene polymorphisms could result from differences between the populations of patients studied, as polymorphic variants might be associated with certain of the patients' characteristics.

In conclusion, polymorphic variants in xenobiotic-metabolism genes, including CYP1A1 and GSTT1, particularly when combined, may increase the risk of adult AML.

FD'A: design and analysis of data, drafting of the article, final approval; MTV: conception and analysis of data, critical revision of article, final approval; FG: analysis of data, critical revision of article, final approval; GM: analysis of data, critical revision of article, final approval; AS, SS, GZ, SH, LP, GL: analysis of data, critical revision of article, final approval. The authors reported no potential conflicts of interest.

This work was supported by grants from M.U.R.S.T (Ministero dell' Universita' e della Ricerca Scientifica e Tecnologica) and A.I.R.C. (Associazione Italiana per la Ricerca sul Cancro).

Manuscript received January 16, 2004. Accepted April 20, 2004.

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