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Association of Glutathione S-transferase Genes (M1 and T1) with the Risk of Acute Myeloid Leukemia in a Moroccan Population

Ait Boujmia Oum Kaltoum*♦ Nadifi Sellama*, Dehbi Hind*, Kassogue Yaya*, Lamchahab Mouna, Quessar Asma****

*Laboratory of Genetics and Molecular Pathology, Medical School, University Hassan II, Casablanca, Morocco
**Department of Onco-Hematology, Ibn Rochd University Hospital, Casablanca, Morocco

Abstract

Background: Acute myeloid leukemia, as most cancers, results from exposure to carcinogens and an impaired inherited individual capacity to eliminate xenobiotics. The present case-control study measures the relationship between glutathione S-transferase (GST) T1 and M1 null genotypes and the risk of acute myeloid leukemia.

Methods: We identified the *GSTT1* and *GSTM1* genotypes by multiplex polymerase chain reaction in 129 acute myeloid leukemia patients and 129 controls.

Results: Individuals that carried *GSTT1* null had a risk of acute myeloid leukemia when compared to *GSTT1* present carriers (OR: 2.80; 95% CI: 1.63-4.80, *P*=0.00036). However, *GSTM1* null did not influence the risk for acute myeloid leukemia (OR: 1.20; 95% CI: 0.72-1.97, *P*=0.53). The combined *GSTT1* null/*GSTM1* present genotype showed an association with the risk for acute myeloid leukemia compared to those that carried both functional genotypes (OR: 8.85; 95% CI: 3.09-23.8, *P*=0.0001). The double null genotype also showed an association with the risk for acute myeloid leukemia (OR: 2.32, 95% CI: 1.15-4.66, *P*=0.019).

Conclusion: Both *GSTT1* null and *GST* double-null genotypes may be risk factors for acute myeloid leukemia. Further studies are needed to confirm these results.

Keywords: *GSTT1*, *GSTM1*, AML, Susceptibility

♦Corresponding Author:
Ait Boujmia Oum Kaltoum, PhD Student
Genetics and Molecular Pathology Laboratory, Medical School of Casablanca, 19 Rue Tarik Ibnou Ziad, BP. 9154 Casablanca, Morocco
Tel: +212661092201
Email: Kaltoum.biologie@gmail.com

Introduction

Acute myeloid leukemia (AML) is a very heterogeneous disease characterized by the proliferation of immature hematopoietic cells called blasts.[1] Clinical and biological signs of AML include anemia, fatigue, infections, bleeding, granulocytopenia, and thrombocytopenia.[2] It is known that the diagnostic and

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therapeutic aspects of AML have improved in recent years. However, the etiology of AML is not clear, but it has been reported that AML results from a complex interaction between factors such as exposure to environmental carcinogens (ionizing radiation, alkylating agents, benzene, and cytotoxic therapy) and individual impaired ability to eliminate toxic substances.[3-5] Indeed, xenobiotic metabolizing enzymes (XME) are known for their participation in the elimination of toxic compounds. Thus, glutathione S-transferases (GSTs) are phase II XME, involved in cellular detoxification by inactivating metabolites from phase I reactions into soluble glutathione, which can be easily eliminated from cells. Therefore, polymorphisms in these enzymes can cause a decrease or a complete absence of enzyme activity, according to the genetic background of the individual, which may cause an individual to develop cancers such as AML.[6,7] Human GSTs are divided into three major families - cytosolic, mitochondrial, and microsomal.[8] The cytosolic family is composed of eight subclasses: alpha, kappa, mu, omega, pi, sigma, theta, and zeta. The mu class and theta class, encoded by the *GSTM1* and *GSTT1* genes are located on chromosome 1p13.3 and chromosome 22q11.23, respectively.[9] The polymorphisms of *GSTT1* and *GSTM1* result in complete deletion of the gene (null allele) which may affect enzyme activity. Therefore, individuals that carry the deleted allele can have a higher risk for development of cancer related to an impaired detoxification capacity.[10] However, several studies have reported an association of the *GSTT1* and *GSTM1* null genotypes and risk of cancers, including colorectal, breast , lung, gastric, head and neck cancers,[11-13] and AML[14-16] whereas other authors have shown no association with the risk of AML.[17, 18] We have performed the present case-control study in a sample of the Moroccan population to test whether the *GSTM1* and *GSTT1* null genotypes contribute to the predisposition of AML.

Materials and Methods

Patients and controls

The local Ethics Committee approved the present study. The study population consisted of 129 patients with AML and 129 healthy controls without any history of cancer. The patients were classified according to World Health Organization 2008 (WHO) criteria[19] and recruited from the Department of Onco-Hematology of the Ibn Rochd University Hospital in Casablanca, Morocco from 2012 to 2015. We extracted demographic and biological data from the data file of each patient. Control subjects were recruited during the same period and consisted of students from the Faculty of Medicine and other volunteers who worked at the same faculty. Each participant accepted and signed the informed consent. Subsequently, we used EDTA tubes to collect 4 ml of peripheral blood from each participant.

Glutathione S-transferase (GST) M1 and GSTT1 genotyping

Genomic DNA was extracted from either blood or bone marrow samples by the salting-out method.[20] DNA purity and concentration was determined using a NanoVue Plus spectrophotometer. The *GSTM1* and *GSTT1* genotypes were detected by multiplex polymerase reaction, in which the *BCL2* gene was used as an internal control. Forward and reverse primers as well as detailed technical aspects have been previously described by Kassogue et al.[21] As seen in Figure 1, the results of the multiplex polymerase reaction showed the following bands for *GSTM1* (219 bp), *GSTT1* (480 bp), and *BCL2* (154 bp).

Statistical analysis

We used either the chi-square or Fisher's exact tests to determine the differences in genotype distributions between cases and controls. Crude odds ratios (OR) with a 95% confidence interval (CI) was calculated. The t-test for independent samples was used to estimate the average age difference between cases and controls. Statistical analysis was performed with the statistical package SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Table1. Demographic and cytogenetic characteristics of acute myeloid leukemia (AML) patients and controls.

Parameters		Case (N=129)	Control (N=129)
Gender N (%)			
	Female	66 (51.2)	79(61.2)
	Male	63 (48.8)	50(38.8)
M:F ratio		0.95	0.63
Age - years (median±SD; range)		38.00±15.67 (3-72)	34.00±15.8 (18-77)
FAB classification N (%)			
	M0	6(4.7)	
	M1	23(17.8)	
	M2	47(36.4)	
	M3	8(6.2)	
	M4	22(17.1)	
	M5	9(7.0)	
	M6	4(3.1)	
	M7	2(1.6)	
Median blasts (% ; range)		82%(10-100)	
Median WBC (G/L; range)		15.15 (0.28-342.82)	
Median platelets (G/L; range)		37(2-400)	
Median hemoglobin (g/dL; range)		7.10 (3-16)	
Karyotype N (%)			
	Normal	64(49.6)	
	Abnormal	57(44.2)	
Risk group			
	Good	24(18.6)	
	Intermediate	84(65.1)	
	Poor	13(10.1)	

N: Number

Results

The present case-control study enrolled 129 AML patients and 129 controls. The clinical and demographic characteristics of the participants are summarized in Table 1. In terms of the French-American-British (FAB) morphological-cytochemical classification, the majority of patients were LAM2 (36.4%), followed by LAM1 (17.8%), and LAM4 (17.1%) while the unclassified cases represented 6.2%. Overall, the *GSTM1* and *GSTT1* distribution was not influenced by biological data such as hemoglobin, platelets, white blood cell (WBC) and blasts, or FAB subtypes. Table 2 shows a comparable distribution of the *GSTM1* null genotype between cases (52.7%) and controls (48.1%). Interestingly, we observed a significantly higher frequency of the *GSTT1* null genotype in cases (40.3%) than in controls (19.4%) with a 2.80-fold increased risk for individuals that carried the *GSTT1* null genotype compared with the *GSTT1* present (95% CI: 1.63-4.80, *P*=0.00036). In Table 3, the interaction between *GSTM1* and *GSTT1* showed that the *GSTT1* present/*GSTM1* null genotype had no association with the risk of AML (OR: 1.68, 95% CI: 0.95-3.03, *P*=0.086) when compared to *GSTT1* present/*GSTM1* present

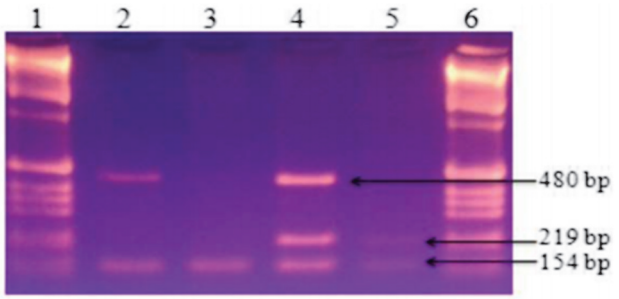

Figure 1. Multiplex PCR analysis of *GSTM1*, *GSTT1* gene polymorphisms. Lane 1; 6: 0.07-12.2 Kbp molecular marker. Lane 2: *GSTT1* present, *GSTM1* null (480 pb). Lane 3: GSTT1 null, *GSTM1* null. Lane 4: *GSTT1* present, GSTM1 present (480-219pb). Lane 5: *GSTT1* null, *GSTM1* present (219 pb). *BCL2* (154 bp) was used as an internal control.
GST: Glutathione S-transferase

Table 2. Genotype distribution of glutathione S-transferases (GST) M1 and *GSTT1* between cases and controls.

Genotype	Patients N (%)	Controls N (%)	OR (95% CI)	*P*-value
GSTM1				
Present	61(47.3)	67(51.9)	Ref.	
Null	68(52.7)	62(48.1)	1.20(0.72-1.97)	0.53
GSTT1				
Present	77(59.7)	104(80.6)	Ref.	
Null	52(40.3)	25(19.4)	2.80 (1.63-4.80)	*0.00036

Ref: Reference; OR:Odds ratio; N: Number; CI:Confidence interval; Stars indicate statistically significant differences.

carriers. However, there a significant association existed with the *GSTT1* null/*GSTM1* null and risk of AML (OR: 2.32, 95% CI: 1.15-4.66, *P*=0.019). Surprisingly, individuals that harbored the *GSTT1* null/ *GSTM1* present genotype had a significant association with an estimated risk of 8.85 for AML (OR: 3.09-23.8, *P*=0.0001).

Discussion

Glutathione S-transferases represent a super-family of detoxifying enzymes that play a key role in the metabolism of a wide range of xenobiotics, including chemical compounds and environmental carcinogens.[7] Therefore, inter-individual genetic variation in the capacity to eliminate xenobiotics associated with long-term exposition to carcinogens could be critical in the development of cancers. In recent decades, many studies have shown the association of GSTs polymorphisms and the risk of developing several types of cancers.[22, 23] In the present case-control study we examined the relationship between *GSTM1* and *GSTT1* polymorphisms to the risk of AML. The most common FAB subtype observed in our study was M2, which confirmed our previous study results.[24] Other authors obtained similar results.[18, 22, 23] We did not find any correlation between the biological parameters and GST genotypes, our results agreed with the observations of Voso et al.[25] who also reported no relationship between the GST genotype and karyotype or FAB subtypes. In contrast, Rollinson et al. reported a correlation.[26] The current study results showed that the *GSTM1* null genotype had no association with the risk of AML (OR 1.2, 95% CI: 0.72-1.97, *P*=0.53). Lemos et al. reported a similar result.[27] On the other hand, Seedhouse et al. found a significantly higher risk of developing AML in patients with the *GSTM1* null genotype (OR, 15.26; 95% CI: 1.83–127.27, *P*=0.01).[28] Yang et al. also showed a significantly higher frequency of the *GSTM1* null genotype in AML patients (62.3%) compared to controls (52.7%, *P*=0.036).[29] The discrepancy between different studies concerning the relation of *GSTM1* null genotype and the risk of AML might be explained by the sample size, geographical differences, and ethnic variations.

Surprisingly, we found a significant association of the *GSTT1* null genotype and an increased risk of developing AML (OR, 2.80; 95% CI: 1.63–4.80, *P*=0.00036). Several studies examined the relationship between *GSTT1* polymorphisms and AML risk, but the results were not conclusive. Ouerhani et al. found no association between the *GSTT1* null genotype and AML risk in Tunisia.[30] Majumdar et al. reported a similar result.[31] However, a positive association between *GSTT1* null genotype and risk of AML has been reported in other studies.[26,32-34] The influence of the *GSTT1* null genotype on the risk of AML could be explained by the diminution of the ability to metabolize or detoxify several carcinogens in individuals lack a functional *GSTT1* gene. The implication of the *GSTT1* null genotype in the risk of AML might be due to long-term exposition to carcinogens by these individuals. We also analyzed the combined effect of *GSTT1*/GSTM1 polymorphisms. The results showed that the *GSTT1* null/*GSTM1* present genotype influenced AML susceptibility (OR: 8.85; 95% CI: 3.09–23.8, *P*=0.0001). This finding might confirm that the *GSTM1* polymorphism did influence the risk of

Table 3. Effect of the combination of glutathione S-transferases (GST) M1and *GSTT1* polymorphisms on the risk of acute myeloid leukemia (AML).

GSTT1	GSTM1	Patients N (%)	Controls N (%)	OR (95% CI)	*P*-value
Present	Present	36(27.9)	62(48.1)	Ref	
Present	Null	41(31.8)	42 (32.5)	1.68(0.95- 3.03)	0.086
Null	Present	25 (19.4)	5 (3.9)	8.85(3.09 - 23.8)	*0.0001
Null	Null	27(20.9)	20(15.5)	2.32 (1.15- 4.66)	*0.019

Ref: Reference;OR:Odds ratio; N: Number; CI:Confidence interval; Stars indicate statistically significant differences.

AML in our population. We observed that the double null genotype of *GSTT1* and *GSTM1* had an association with AML risk. Our results agreed with previous studies by Arruda et al., Haase et al., and He et al. who demonstrated an association between the double null genotype and AML risk.[17,18,35] In contrast, Crump et al. reported that there was no association between the null *GSTT1* and the null *GSTM1* genotypes and the risk of AML.[36]

Conclusion

The present study suggests that the *GSTT1*, but not the *GSTM1* polymorphism is associated with AML susceptibility in our population. Further studies with large population size are necessary to confirm the association between GST polymorphisms and AML risk.

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Conflict of Interest

No conflict of interest is declared.

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