Electronic journal of oncology

Bulletin du Cancer
Société Française du Cancer

Volume 98 • N° 12 • décembre 2011

Influence of genetic polymorphisms of xenobiotic metabolizing enzymes on the risk of developing leukemia in a Tunisian population

Slah Ouerhani[1], Mohamed Ali Nefzi[1], Samia Menif[1], Inès Safra[1], Kais Douzi[1], Chaker Fouzai[1], Ghofrane Ben Ghorbel[1], Islem Ben Bahria[2], Amel Ben Ammar Elgaaied[2], Salem Abbes[1,2]

[1] Pasteur Institute of Tunis, laboratory of molecular and cellular haematology, BP 74, 13, place Pasteur, Tunis le Belvédère, 1002 Tunis, Tunisia
<slah_mekni@yahoo.fr>

[2] Laboratory of Genetic, Immunology and Human Pathology, Faculty of Sciences of Tunis, El Mannar I. 2092 Tunis, Tunisia

Article received on February 18, 2011,
accepted on July 8, 2011
Reprint: S. Ouerhani

To cite this article: Ouerhani S, Nefzi MA, Menif S, Safra I, Douzi K, Fouzai C, Ben Ghorbel G, Ben Bahria I, Ben Ammar Elgaaied A, Abbes S. Influence of genetic polymorphisms of xenobiotic metabolizing enzymes on the risk of developing leukemia in a Tunisian population. *Bull Cancer* 2011 ; 98 : E95-E106.
doi : 10.1684/bdc.2011.1502.

Abstract. Leukemia is a type of cancer of the blood or bone marrow that is characterized by an abnormal increase of white blood cells. Leukemia is clinically and pathologically subdivided into a variety of large groups. The risk of developing leukemia may be influenced by polymorphisms of xenobiotic metabolizing enzymes. In this work, we conduct a case-control study to assess the impact of polymorphisms in *GSTM1*, *GSTT1* and *NAT2* genes on the risk of developing leukemia. Our data have shown that *GSTM1*0* and *GSTT1*0* were respectively associated with 2.05 and 4.36 increased risk for acute lymphoblastic leukemia (ALL). We have also shown that *GSTM1*0* and *GSTT1*0* act additively to increase the risk for ALL. Indeed, patients harbouring the "*GSTM1*0/GSTT1*0*" genotype were at 11.81-fold increased risk for developing ALL ($P = 2\ 10^{-5}$). The risk for developing acute myeloid leukemia (AML) increases on patients with "rapid or intermediate *NAT2* genotypes". Finally, the comparison of leukemia subgroups according to *GSTM1*, *GSTT1* and *NAT2* genotypes, suggests that leukemogenesis of different leukemia subgroups is very distinct. In conclusion, our findings suggest that leukemogenesis is associated with carcinogen metabolism and consequently related to environmental exposures. ▲

Key words: xenobiotic, leukemia, polymorphism, GST, NAT, susceptibility

Introduction

Leukemia is a type of cancer of the blood or bone marrow that is characterized by an abnormal increase of white blood cells. Leukemia is clinically and pathologically subdivided into a variety of large groups. The first division is between its acute and chronic forms. Additionally, the diseases are subdivided according to the kind of blood cell that is affected. This split divides leukemia into lymphoblastic and myeloid leukemia. The risk of developing leukemia may be influenced by xenobiotic metabolizing enzymes. The metabolism of xenobiotic is divided into two enzymatic pathways, including activation into electrophilic compounds by phase I reactions, followed by biotransformation during phase II reactions. Among the xenobiotic metabolism enzymes, the N-acetyltransferases (NAT) and glutathione transferases (GST) enzymes, a phase II enzymes, are of interest to haematologists because they metabolize many products such as chemotherapeutic and carcinogens agents and they provide targets for antitumour drug therapies. Among the phase II enzymes the GSTM1, GSTT1 and NAT2 enzymes have been evaluated as risk factors for cancers in a number of studies. The NAT2 enzyme catalyses the transformation of aromatic and heterocyclic amine presents in tobacco and metabolizes a range of drugs such as isoniazid and sulfamethoxazole. This enzyme is encoded by a gene located on chromosome 8 and has an open reading frame of 870-base pair [1]. Sixty-two alleles and 14 clusters were described in open reading frame for *NAT2* gene [2, 3]. The *NAT2*4* allele has historically been designated "wildtype" and was associated with

doi : 10.1684/bdc.2011.1502

a rapid (or extensive) enzyme activity. The *NAT2*5*, *NAT2*6*, *NAT2*7*, *NAT2*12D*, *NAT2*14*, *NAT2*17*, *NAT2*19* alleles have been correlated with decreased NAT2 enzyme activity. Of these, *NAT2*5*, *NAT2*6* and *NAT2*7* cluster defined by 341T > C, 590G > A, and 857G > A SNPs were the most described and for which an interethnic and intraethnic variability has been reported [2-4]. Although the slow *NAT2* acetylation genotypes have been implicated with altered susceptibility in a number of cancers [5-7], studies focusing on their relationship with the risk of leukemia have produced conflicting results. While, Zanrosso *et al*. have shown that slow *NAT2* acetylators haplotypes were associated with 8.90-fold increased risk of infantile leukemia [8], Vineis *et al*. did not detect any association between *NAT2* genotype and leukemia [9]. In contrast, Lemos *et al*. suggested an association of extensive metabolism with an increased risk of leukemia [10].

The mammalian cytosolic GSTs are dimeric enzymes with subunits of 199-244 amino acids in length and catalyses conjugation of electrophilic substrates with glutathione, usually resulting in detoxification of reactive intermediates [11, 12]. Lack of the encoded enzymes has been identified in both *GSTM1* and *GSTT1* loci. The absence of GSTM1 and GSTT1 enzyme activities is due to a homozygous deletion in the *GSTM1* and *GSTT1* gene (*GSTM1*0* and *GSTT1*0*). An interethnic variability has been described for both *GSTM1* and *GSTT1* homozygous deletions [13, 14]. Subjects lacking *GSTM1* and *GSTT1* are at increased risk of environmentally-related cancer such as bladder and lung cancer [15-17]. For leukemia, some studies have suggested that *GSTT1* null genotype was associated with a significant increase in the risk of chronic myeloid leukemia (CML) [18]. Other studies, have shown that *GSTT1* null genotype was shown to be risk factors for both acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) [19, 20]. Considering *GSTM1*0* genotype, while some studies failed to detect any association between this polymorphism and risk for leukemia development [12, 21], others have suggested that the risk of ALL is doubled in patients who carry the *GSTM1* deletion [22]. Moreover, a recent meta-analyse has suggested a statistically significant increased risk associated with *GSTM1* for the ALL patients [23].

Considering the role in detoxification played by these enzymes, the existence of common polymorphisms and previous report of their association with other cancers, we aimed at determining whether there is any association between these polymorphisms and susceptibility to leukemia. Therefore, we have extracted DNA from patients and healthy subjects to investigate the effect of the homozygous deletions on *GSTM1* and *GSTT1* genes and *NAT2*5* (341T > C; rs1801280); *NAT2*7* (857G > A; rs1799931) and *NAT2*14* (191G > A; rs1801279) SNPs on leukemia susceptibility.

Patients and methods

Subjects

Our analysis included 309 controls and 193 patients. Among patients, 50 were diagnosed with ALL, 47 with AML, 49 with CML and 47 with chronic lymphocytic leukemia (CLL). In the ALL group, only adults were analysed and children were excluded. All patients were recruited at the first diagnosis from the laboratory of molecular and cellular haematology from Pasteur Institute of Tunis, Tunisia. The diagnosis of leukemia subgroups was based on the standard clinico-haematological criteria, immunophenotypage and the presence of Philadelphia chromosome and/or *BCR-ABL* fusion gene (for the CML).

The control group consisted of healthy unrelated volunteers without a medical history of cancer. Controls were recruited from different geographic origins, among them 50.45% were men and the mean age was 48.35 ± 13.08 years. The control group was approximately matched for gender proportion, geographic origin and age range to those in the case group. Under informed consent, peripheral blood samples were collected into tubes with EDTA.

DNA preparation and genotyping

Blood or bone marrow was collected into EDTA-containing tubes and genomic DNA was extracted by the conventional phenol-chloroform method and by the TRizol® protocol. The quality of genomic DNA was controlled by electrophoresis on a 1% agarose gel stained with ethidium bromide. *GSTM1* and *GSTT1* null genotypes (*GSTM1*0* and *GSTT1*0*) were identified using a multiplex-polymerase chain reaction (PCR)-based method as described by Arand *et al*. [24]. For *NAT2*, a PCR was carried out as described by Hsieh *et al*. [25]. The whole intronless *NAT2* gene resulted in a 1093-base pair amplificate. It was then digested at 37 °C overnight with 5 U of *Kpn*I, 10 U of *Bam*HI, and 10 U of *Alu*I, to reveal