

Special Article - Acute and Chronic Myeloid Leukemia

Change of COMT Val158Met Genotype in Tumoral B Cells of a Chronic Lymphocytic Leukemia Patient: A Case Report

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In this case report, a Chronic lymphocytic leukemia (CLL) female patient with a heterozygous polymorphism in the catechol-O-methyltransferase (COMT) gene is described. This functional change in the COMT enzyme Val158Met leads to a decreased efficacy to O-methylate different endogenous and exogenous compounds containing catechol structure, including hydroxylated estradiol intermediates, catecholamines and certain dietary flavonoids. The specific change found in tumoral B-cells as compared to the genomic DNA from the non-B fraction of peripheral blood mononuclear cells of the patient, could indicate that Val158Met might play some role in the pathogenesis of CLL that certainly needs further investigations. The possible application of this polymorphism as a potential biomarker of CLL is also worth of further large-scale case-control studies.

Keywords: Biomarker; Catechol-O-methyltransferase; Chronic lymphocytic leukemia; O-methylation**Case Presentation**

The venous blood sample was taken from a 64-year-old woman within a regular check-up. The patient had a 7-year history of chronic lymphocytic leukemia (B-CLL; Rai stage 1 and Binet stage A disease) and her hematological status was stable. As patient did not have any B symptoms, anemia or thrombocytopenia and she did not need any specific treatment according to the current CLL management approaches. Laboratorial findings revealed the white blood cell count (WBC) of $30 \times 10^9/L$ with lymphocyte count of $25 \times 10^9/L$. Phenotype of the lymphocytes was determined as CD45+, CD19+, CD20+, CD5+, CD23+, CD43+, CD38-, FMC7-, CD79b-. Lymph nodes were observed only in cervical region with the diameter less than 1 cm. Fluorescence in situ hybridization revealed the presence of a monoallelic 13q deletion. The IGHV status of the patient as well as the expression of ZAP70 was not studied.

Peripheral blood mononuclear cells (PBMCs) were further separated from the whole blood sample using a density gradient technique (Ficoll-Paque™ Premium, GE Healthcare). B cells were isolated from PBMCs by the human B-CLL Cell Isolation Kit from MACS Miltenyi Biotec. DNA was separated from whole blood sample, B cells and non-B fraction of PBMCs using QIAamp DNA Mini Kit (Qiagen) and the respective concentrations were determined by NanoDrop 2000C spectrophotometer (Thermo Scientific). Because of the recent increasing interest in possible role of phase II enzymes and their genetic variants in carcinogenesis [1], the fourth exon of catechol-O-methyltransferase (COMT) gene was amplified by polymerase chain reaction (PCR) and the COMT Val158Met (rs4680, G>A) genotype was determined by restriction analysis using FastDigest NlaIII (HinIII, Thermo Scientific) as the restriction enzyme. Standard gel picture of three different genotypes

(Val/Val, GG; Met/Met, AA and Val/Met, GA) is presented in the Figure 1A. The genotyping results of the patient demonstrated GG for the normal healthy non-B PBMCs but the genotype of tumoral B cells was changed to heterozygous GA (Figure 1B).

Discussion

B-cell chronic lymphocytic leukemia (B-CLL) is the most frequent form of leukemia in adult population of the Western world being characterized by the progressive accumulation of mature non-functional B lymphocytes with dysregulated apoptotic pathways. Current conventional therapies have been focused mostly on controlling of the symptoms rather than curing of patients [2,3]. Also, the metabolism of chronic lymphocytic leukemia cells is still relatively less studied [4,5].

Catechol-O-methyltransferase (COMT, EC 2.1.1.6) is a significant phase II detoxification enzyme that catalyzes O-methylation reaction by relocating a methyl group from S-adenosylmethionine to one of the hydroxyl groups on substrate with catecholic structure [6-11]. This ubiquitous enzyme can be found in almost all mammalian tissues and it has a wide range of substrates, including endogenous catecholamines and catechol estrogens, but also exogenous compounds, such as certain dietary flavonoids [6-8,11,12]. The COMT gene is located on chromosome 22q11.2 and it encodes two different enzyme forms, a 221 amino acid length cytoplasmic protein and a membrane-bound form that contains additional 50 amino acid residues [6,7,13,14]. The most of the COMT occurring in human tissues is cytoplasmic [6,8,10,15,16]. However, COMT enzyme is polymorphic and its activity has significant differences among individuals [8,14-20]. The single nucleotide polymorphism (SNP) G>A (rs4680) leads to an amino acid change of Val to Met in the position 108/158 in cytoplasmic/ membranous enzyme form,

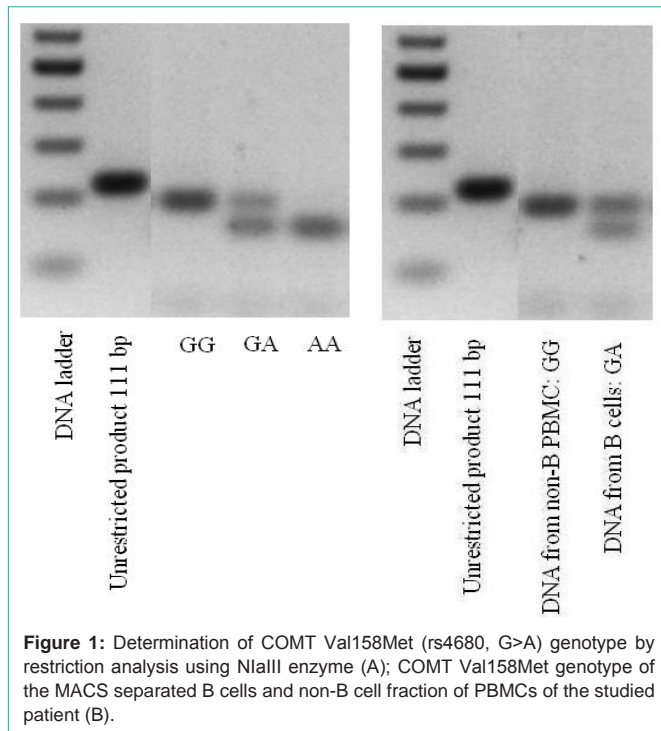


Figure 1: Determination of COMT Val158Met (rs4680, G>A) genotype by restriction analysis using NlaIII enzyme (A); COMT Val158Met genotype of the MACS separated B cells and non-B cell fraction of PBMCs of the studied patient (B).

respectively, and is generally known as Val158Met. This SNP has important (two- to fivefold) impact on the enzymatic activity as those individuals homozygous for the Val (GG) have high activity enzyme variant, those with Met/Met (AA) possess low activity protein and heterozygous genotype carriers (Val/Met, GA) have the enzyme form with intermediate activity [7,12,14,17,19-28]. It means that alteration in the respective genotype leads to the change in efficacy of O-methylation of different endogenous and exogenous catecholic compounds (including for example catechol estrogens, but also flavonoids like quercetin, fisetin and different flavanols) and therefore also to the increased amounts of circulating catechols with significantly different bioactivities compared to their O-methylated metabolites.

In this case report, we demonstrate the change of rs4680 genotype (GG>GA) in tumoral B cells of a CLL patient as compared to her genomic DNA separated from the healthy non-B PBMCs, pointing to the respective decrease in enzymatic activity in malignant cells. This alteration cannot be caused by the treatment modalities as the blood sample was taken when the patient has not received any therapies yet. We hypothesize that this genetic change may have some role in the pathogenesis (initiation and/or progression) of CLL. It is well known that hydroxylated intermediates of estradiol (2- and 4-hydroxylated estradiols) can be oxidated to highly reactive semiquinones and quinones which may induce DNA damage and cause therefore cancer development [15-21,25,29-32]. These harmful effects are contravened by detoxification enzymes, mainly by COMT that catalyzes O-methylation of different catechols and facilitates their excretion from the body [14,20]. It is clear that changes in the enzymatic activity of COMT caused by Val158Met polymorphism may be implicated in carcinogenesis. In accordance with this, the results of the current case report show that the higher enzymatic activity could function as a potential protective factor against CLL. Unfortunately, differently

from various solid tumors, there are still no epidemiological studies available about the possible role of COMT variants in the risk of CLL, although a report of Skibola, et al. indicated an increased susceptibility of American women with low activity enzyme variant (Met/Met, AA) toward non-Hodgkin lymphoma [33,34]. We are currently completing a study about the distribution of COMT Val158Met genotypes in the Estonian CLL patients in comparison with the age- and gender-matched population-based controls and the results of this work will be summarized in the near future.

In conclusion, we described in this case report a CLL patient with a functional (heterozygous) polymorphism in the COMT gene in her malignant B cells that can be important in the pathogenesis of CLL. This finding suggests that further studies about the COMT Val158Met as a possible biomarker for CLL are needed to confirm or disprove our hypothesis. Nevertheless, considering that the current treatment options of CLL often do not provide a final deletion of malignant clone, development of novel treatment strategies are of great importance.

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