Polymorphisms in COMT and SULT1A1 Genes and Chronic Lymphocytic Leukemia Risk in Estonia

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Abstract

Objectives: Studies investigating associations between common polymorphisms in phase II metabolic enzymes COMT and SULT1A1 and the risk of various cancer types have revealed inconsistent and controversial results, with no attention turned to date to the most common adult leukemia type, i.e., chronic lymphocytic leukemia.

Methods: In this small case-control study with 47 cases and 50 controls, the role of two functional polymorphisms, Val158Met in COMT gene and Arg213His in SULT1A1 gene affecting the activity level of respective enzymes, was studied on the susceptibility to chronic lymphocytic leukemia in an Estonian cohort.

Results: Although statistically non-significant (p>0.05), the suggestive reduction in disease risk observed with low activity enzyme variants could indicate the involvement of O-methylation and sulfation of various endogenous and exogenous substances in the process of leukemogenesis. The odds ratio (OR) for Met158Met genotype of COMT was 0.60 with 95% confidence interval (95% CI) 0.20-1.82 compared to the wild type Val158Val genotype and the OR for His213His genotype of SULT1A1 was 0.58 with 95% CI 0.20-1.71 compared to the wild type Arg213Arg genotype.

Conclusion: Further large-scale studies are highly needed to confirm or disprove the findings of the present study and determine genetic risk factors for chronic lymphocytic leukemia.

Keywords: Chronic lymphocytic leukemia, O-methylation, phase II enzyme, single nucleotide polymorphism, sulfate conjugation


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Cchoric lymphocytic leukemia (CLL) is a heterogeneous malignancy with a high variability in clinical presentations affecting mainly the elderly with mean age at diagnosis of nearly 70 years.[1,2] With annual incidence of 3-5 cases per 100000 persons, CLL is the most common leukemia in adults of the Western world revealing about two-fold higher incidence rate in males than females.[3,4] This disease is characterized by the clonal proliferation of the mature CD5/CD19 lymphocytes in the bone marrow, blood, and lymphoid tissues, such as lymph nodes and spleen.[2] Despite several efforts made in the last decades in evolution of new treatment strategies, including development of anti-CD20 antibodies-based immunochemotherapy options, Bruton’s tyrosine kinase inhibitors and phosphoinositide 3-kinase inhibitors, CLL is still considered an incurable disease.[1,2,4] Therefore, seeking further and more effective approaches are highly needed to attain the eventual goal of curing CLL. In parallel, development of strategies to select at-risk individuals using appropriate biomarkers is also important.
It has been previously suggested that genetic changes in metabolic enzymes can be potentially important in carcinogenesis process and certain polymorphisms in genes encoding these enzymes might be considered as disease biomarkers. However, only very few association studies are still focused on hematological malignancies.\textsuperscript{[5, 6]}

Catechol-O-methyltransferase (COMT, EC 2.1.1.6) is a phase II enzyme that metabolizes various compounds with catecholic structure.\textsuperscript{[6]} Dr. Julius Axelrod described this enzyme first in 1958\textsuperscript{[7]} and nowadays it is generally accepted that COMT plays an important role in the O-methylation and detoxification of various catechols, including endogenous catechol estrogens and catecholamines, but also catechol-containing exogenous substances.\textsuperscript{[8–11]} This enzyme catalyzes the transfer of a methyl moiety from S-adenosylmethionine to one of the hydroxyl groups on catechol compound.\textsuperscript{[12, 13]} A single COMT gene encodes two forms of COMT enzymes, a membrane-bound protein with 271 amino acid residues and a soluble cytoplasmic protein with 221 amino acid residues, whereas the majority of COMT is present in the soluble form in human tissues apart from brain where COMT exists in membranous variant.\textsuperscript{[14–16]} Although expression of COMT is highest in kidney and liver, it can be detected practically in all human tissues.\textsuperscript{[12, 17]} In 1995, a genetic change of guanine (G) to adenine (A) in the exon 4 of COMT gene was described by Lotta et al resulting in amino acid replacement of valine (Val) to methionine (Met) in codon 158 in membrane-bound variant and at position 108 in soluble enzyme form.\textsuperscript{[18–21]} This single nucleotide polymorphism (SNP; rs4680) was found to control the activity of COMT enzyme inducing high-, intermediate- and low-activity enzyme phenotypes, whereas the change Val158Met has been related to two- to fivefold reduction in the activity level of COMT.\textsuperscript{[15, 20, 22, 23]} Thus, subjects carrying two Met alleles (Met158Met) exhibit considerably reduced ability to O-methylate various catechols resulting in an increase in circulating catechol-containing substances compared to the wild type individuals with two Val alleles (Val158Val); heterozygotes (Val158Met) have intermediate enzyme activity.\textsuperscript{[12, 17, 24, 25]}

Sulfotransferase (SULT) 1A1 (EC 2.8.2.1) is another phase II enzyme that mediates sulfation of various compounds with phenolic structure.\textsuperscript{[5]} This enzyme catalyzes the transfer of sulfonyl group from 3’-phosphoadenosine-5’-phosphosulfate to a wide variety of phenolic substances, including endogenous estrogens and thyroid hormones, but also different exogenous agents.\textsuperscript{[5, 26]} The SULT1A1 enzyme is a 295 amino acid protein being expressed in the majority of human tissues.\textsuperscript{[26–28]} The most common polymorphic change in SULT1A1 (rs9282861) consists of G to A nucleotide transition at position 638 in exon 7 leading to an arginine (Arg) to histidine (His) replacement at amino acid 213.\textsuperscript{[29–31]} At that, subjects homozygous to variant His alleles (His213His) reveal only approximately 15% of enzyme activity compared to those with wild type SULT1A1 form (Arg213Arg).\textsuperscript{[28, 31, 32]} As sulfation results in more polar compounds with promoted elimination from the human body, this process is generally considered a detoxification pathway.\textsuperscript{[33, 34]}

To investigate the possible involvement of metabolic enzymes in development of chronic lymphocytic leukemia, a small-scale case-control study on association between functional polymorphisms in COMT and SULT1A1 enzymes and the risk of CLL was performed in Estonian population.

### Methods

#### Study Subjects

This case-control study was conducted in Tartu, Estonia. Cases were individuals (n=47) with clinically confirmed diagnosis of CLL treated in the Clinic of Hematology and Oncology, Tartu University Hospital. Controls (n=50) were recruited from medical staff as well as healthy subjects visiting the same hospital for examination of hereditary cancer risk. All participants were older than 45 years in the time of blood collection and were recruited during 2011-2016. The research protocol was approved by the Research Ethics Committee of the University of Tartu (Approval 245/T-4). A written informed content was obtained from each subject.

#### Isolation of Peripheral Blood Mononuclear Cells (PBMC), B Cells and Non-B Cells

Peripheral blood samples (10 ml) were collected from all CLL patients and healthy volunteers. Mononuclear cells were isolated from heparinized venous blood by density-gradient centrifugation by using Ficoll separation method. Ficoll-PaqueTM Premium was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Only freshly isolated normal PBMCs were used for further separation of B cells and non-B cells. B cells were isolated from PBMCs using the human B-CLL Cell Isolation Kit obtained from MACS Miltenyi Biotec (Auburn, CA, USA). At that, the fraction of non-B cells was also collected.

#### DNA Separation and Genotyping

As tumoral B cells can contain CLL specific genetic changes, the genomic DNA was isolated from non-B cell fraction of PBMCs using QIAamp DNA Mini Kit protocol (Qiagen, Inc., Chatsworth, CA, USA). The PCR reaction was carried out in the volume of 20 μl, containing 1 μl of genomic DNA, 4 μl of 5x HOT FIREPol Blend Master Mix Ready to Load (Solis Bioodyne, Tartu, Estonia) and 0.8 μl of 10 μM primer solutions. A 111 bp fragment of COMT was amplified by using the fol-
lowing primers: forward 5´-GGCTCATACCATCGAGATCAA-3´ and reverse 5´-CCAGGTCTGACACCGGTTCA-3´. A 287 bp fragment of SULT1A1 was amplified by using the following primers: forward 5´-TTGGCTCTGCAGGGTTTCTA-3´ and reverse 5´-GGAGATGCTGTGGTCCATGA-3´. PCR primers were synthesized in Metabion (Planegg/Steinkirchen, Germany). The PCR reaction was performed as follows: 1 cycle of 5 min predenaturation at 94 0C, then 45 cycles of 30 s at 95 0C, 30 s annealing at 61 0C, 30 s extension at 72 0C, and followed by 1 cycle of 5 min extension at 72 0C. The correctness of PCR products was analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide.

Amplified fragments of COMT and SULT1A1 were cleaved by HinII (NlaIII) and BfoI (Haell) restriction enzymes, respectively (Thermo Fisher Scientific Inc., Lithuania). These reactions were performed in the volume of 30 μl containing 2 μl of 10x FastDigest Green Buffer (Thermo Fisher Scientific Inc., Lithuania), 10 μl amplified PCR product and 1 μl FastDigest restriction enzyme. Incubations were conducted at 37 0C for 1 hr and 37 0C for 30 min for HinII and BfoI with following inactivation at 80 0C for 5 min and 65 0C for 10 min, respectively. Products of restriction fragment lengths were separated on 2.4% agarose gel in TAE buffer (Tris-acetate-EDTA) at ambient temperature using GeneRuler 50 bp DNA ladder as marker (Thermo Fisher Scientific Inc., Lithuania).

DNA sequencing reactions were performed in the core laboratory of Estonian Biocentre by Sanger sequencing method using BigDye Terminator v3.1 enzyme mixture. Examples of DNA genotyping for Val158Met in COMT gene and Arg213His in SULT1A1 gene are demonstrated in Figure 1 and Figure 2, respectively.

Statistical Analysis

The frequency distributions of polymorphic genotypes of COMT Val158Met and SULT1A1 Arg213His for CLL patients and healthy controls were calculated and tabulated. To determine differences in the genotypic distributions between cases and controls, Chi-square test was performed. For each polymorphic site, odds ratio values (ORs) along with 95% confidence intervals (95% CIs) were determined using unconditional logistic regression model. Subjects with wild type genotypes (COMT Val158Val, SULT1A1 Arg213Arg) were used as reference groups for comparison of risk allele containing genotypes. P value below 0.05 was considered statistically significant.

Results

The study population included 47 CLL cases and 50 controls that were all analyzed concerning the genotypes in
Table 1 represents the genotypic frequencies of COMT and SULT1A1 polymorphisms. These results showed the distribution of COMT Val158Met genotypes among CLL cases of Val/Val 30%, Val/Met 51%, Met/Met 19% and controls 28%, 42%, 30%, respectively. The distribution of SULT1A1 Arg213His genotypes among CLL cases were Arg/Arg 32%, Arg/His 47%, His/His 21% and among controls 28%, 40%, 32%, respectively. Although no statistically significant associations revealed, individuals with low methylation and sulfation activities exerted a suggestive inverse association with CLL risk (OR=0.60, 95% CI 0.20-1.82 for COMT Met158Met and OR=0.58, 95% CI 0.20-1.71 for SULT1A1 His213His), as compared to individuals carrying wild type genotypes, i.e., Val158Val for COMT and Arg213Arg for SULT1A1.

**Discussion**

Due to a small general population in Estonia, the number of CLL cases involved in this work was also rather limited being reflected in the odds ratio measures with wide confidence intervals. However, the distribution of COMT genotypes among controls (28% Val158Val, 42% Val158Met, 30% Met158Met) is similar to the values described previously for Caucasian subjects (~25% Val158Val, ~50% Val158Met, ~25% Met158Met) adding validity to the results of the current work.[6, 35]

One of the most important functions of COMT and SULT1A1 enzymes in the human body is metabolization of estrogen to methoxy estrogens and estrogen sulfates by inactivating the potentially harmful intermediate semiquinones and quinones formed from catechol estrogens during their oxidation.[6, 19, 36–38] Lowered activity of detoxification processes due to functional polymorphisms in COMT and SULT1A1 enzymes can lead to accumulation of mutagenic and carcinogenic catechol estrogens and therefore elevate the cancer risk.[6, 20, 35, 39] Indeed, experiments with the Syrian hamsters revealed an increased renal tumorigenesis by suppressing the activity of COMT.[40] In this context, the results of the current work, showing a suggestive, still statistically non-significant, 40% and 42% decrease in CLL risk with low activity enzyme variants COMT Met158Met and SULT1A1 His213His respectively, are somewhat unexpected. Although it is possible that this outcome was caused by chance as the numbers of study subjects with corresponding genotypes were very small, the lowered risk can still be explained by several factors. First, albeit O-methylation of catechol estrogens is considered a detoxification pathway, elevated amounts of 2-methoxyestradiol has been recently found to produce breaks in chromosome and aneuploidy contributing thus to development of neoplasias.[41] Second, besides well-known function in detoxification of various cell-damaging substances, sulfation can also bioactivate several promutagens and carcinogens from food and environment, such as heterocyclic amines and polycyclic aromatic hydrocarbons, leading to a potential DNA damage.[5, 42–49] These dual actions can also provide some explanations to the inconsistent and controversial findings of epidemiological studies about associations between COMT Val158Met and SULT1A1 Arg213His genotypes and susceptibility to different cancer types indicating that these relationships can be much more complex and depend probably on numerous endogenous and exogenous determinants, such as age, gender, ethnicity, level of physical activity, obesity, menopausal status, age at menarche, number of full term pregnancies, family history of certain malignant disorders, but also exposure to contraceptives and hormone replacement therapy, tobacco smoke or other environmental pollutants.[5, 6, 9, 50–55] Unfortunately, the small number of study subjects in this work did not allow us to make any further stratification analyses for susceptibility to CLL.

Furthermore, several plant-derived dietary flavonoids, such as flavonols quercetin, myricetin and fisetin, flavon

<table>
<thead>
<tr>
<th>COMT Val158Met</th>
<th>47 Cases (%)</th>
<th>50 Controls (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val/Val (high)</td>
<td>14 (30)</td>
<td>14 (28)</td>
<td>1 (Ref)</td>
<td></td>
</tr>
<tr>
<td>Val/Met (intermediate)</td>
<td>24 (51)</td>
<td>21 (42)</td>
<td>1.14 (0.44-2.94)</td>
<td>0.78</td>
</tr>
<tr>
<td>Met/Met (low)</td>
<td>9 (19)</td>
<td>15 (30)</td>
<td>0.60 (0.20-1.82)</td>
<td>0.37</td>
</tr>
<tr>
<td>Val/Met, Met/Met</td>
<td>33 (70)</td>
<td>36 (72)</td>
<td>0.92 (0.38-2.21)</td>
<td>0.84</td>
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<table>
<thead>
<tr>
<th>SULT1A1 Arg213His</th>
<th>47 Cases (%)</th>
<th>50 Controls (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg (high)</td>
<td>15 (32)</td>
<td>14 (28)</td>
<td>1 (Ref)</td>
<td></td>
</tr>
<tr>
<td>Arg/His (intermediate)</td>
<td>22 (47)</td>
<td>20 (40)</td>
<td>1.03 (0.40-2.65)</td>
<td>0.84</td>
</tr>
<tr>
<td>His/His (low)</td>
<td>10 (21)</td>
<td>16 (32)</td>
<td>0.58 (0.20-1.71)</td>
<td>0.32</td>
</tr>
<tr>
<td>Arg/His, His/His</td>
<td>32 (68)</td>
<td>36 (72)</td>
<td>0.83 (0.35-1.98)</td>
<td>0.67</td>
</tr>
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</table>
luteolin and tea flavanols (catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate) also contain catecholic structure representing thus substrates for O-methylation by COMT. These and other flavonoids with general polyphenolic structure are also metabolized by SULT1A1-catalyzed sulfation reaction in the small intestine and liver. Depend on the dietary habits, humans consume up to 1 g of flavonoids per day, presenting in fruits and vegetables, nuts, spices, medicinal herbs, but also plant-based beverages like cocoa and tea. Numerous in vitro works with cell lines and in vivo studies with animal models have demonstrated that these plant secondary metabolites exert various anticancer properties, such as antioxidant, antiproliferative, proapoptotic, antiinvasive, antiangiogenic and antimetastatic effects. However, compared to the parent flavonoids originally present in plant-based food items, anticancer potential of their O-methylated or sulfated conjugates as the major metabolites entering bloodstream is reported to be considerably less efficient. Although the current knowledge about anticancer action of COMT- and SULT1A1-mediated derivatives of flavonoids is still rather limited, it is possible that lower efficacy of O-methylation and sulfation processes caused by polymorphisms in these enzymes can provide somewhat higher levels of intact flavonoids entering circulation and reaching target tissues to exert more protection against initiation and development of chronic lymphocytic leukemia.

According to the best knowledge of authors, there are no studies performed to date about the role of COMT and SULT1A1 polymorphisms in susceptibility to CLL. For COMT Val158Met, Skibola et al. demonstrated a 1.6-fold increase in the risk of non-Hodgkin lymphoma among American women with two Met alleles (Met158Met) compared to their counterparts homozygous to Val alleles (Val158Val). Concerning SULT1A1, no results about the possible role of Arg213His polymorphism on hematological malignancies have been reported.

The strengths of the current work included ethnical homogeneity of the study population and obviating the possibility for detection of genetic changes formed in any stages during carcinogenesis as tumoral B cells were eliminated from peripheral blood mononuclear cells before separation of genomic DNA. Indeed, we have previously shown that Val158Met polymorphism in COMT gene can be specific for tumoral B cells and possibly involved in the progression of CLL. However, this study had also several limitations, including small number of subjects and a possible selection bias. As controls, a mixed cohort of medical staff and healthy individuals visiting hospital for risk estimation of hereditary cancers was used. The possibility that distribution of COMT and SULT1A1 genotypes among people with family history of neoplasms might differ from general population can not be fully excluded. Also, we can not rule out the possibility that some unknown factors could play role in associations between studied gene variants and tumorigenesis risk.

This is the first study presenting results on associations between functional polymorphisms in COMT and SULT1A1 metabolic enzymes and the risk of CLL. Although statistically non-significant, the suggestive reduction of CLL risk related to low activity enzyme variants certainly needs further investigations with larger study cohorts allowing also stratifications by potentially important endogenous and environmental factors. At that, dietary choices and preferences of plant-based food products rich in natural flavonoids may prove to be crucial. In parallel, expansion of in vitro and in vivo studies on antileukemic action of methylated and sulfated metabolites of dietary polyphenols is equally important to delineate possibilities for possible prevention of CLL in the future.

Disclosures

Ethics Committee Approval: Ethics Committee Approval number is: 245/T-4
Peer-review: Externally peer-reviewed.
Conflict of Interest: None declared.


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