



## CYP2D6 gene amplification and the risk of acute myeloblastic leukemia

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### Abstract:

The aim of this work was to evaluate whether patients with acute myeloblastic leukemia (AML) differ from healthy persons in their CYP2D6 genotype. The study was carried out in 34 patients with *de novo* acute myeloblastic leukemia before chemotherapy and 64 healthy persons as a control group. Mutation in the CYP2D6\*3 and CYP2D6\*4 alleles was analyzed by polymerase chain reaction amplification and restriction fragment length polymorphism (PCR-RFLP) techniques. Genotyping for the CYP2D6 gene amplification was performed by polymerase chain reaction (PCR) amplification techniques. The frequency of CYP2D6\*1, CYP2D6\*3, CYP2D6\*4, CYP2D6\*1xn, CYP2D6\*4xn alleles among 64 genotyped healthy persons was 75.0%, 1.5%, 22.7%, 0.8% and 0.0%, respectively and among 34 genotyped patients with acute myeloblastic leukemia: 69.1%, 1.5%, 10.3%, 17.6% and 1.5%, respectively. Statistically significant differences were detected in the gene amplification ( $p < 0.0001$ ,  $\chi^2 = 14.6$ ) and CYP2D6\*4 allele ( $p = 0.05$ ,  $\chi^2 = 3.74$ ) frequency between the group of patients with acute myeloblastic leukemia and healthy persons. The odds ratio for ultra rapid metabolizers (UM) was statistically significant, it was about 25-fold greater in the group of patients with acute myeloblastic leukemia than in the group of healthy persons. Our findings indicated an overrepresentation of UM and an underrepresentation of CYP2D6\*4 allele among patients with acute myeloblastic leukemia.

### Key words:

CYP2D6, amplification, acute myeloblastic leukemia, polymorphism, ultra rapid metabolizers

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## Introduction

In the last few years, numerous observations have elucidated the role of hereditary factors in the susceptibility to some diseases. The relationship between genetically determined polymorphic metabolism of exogenous substances by oxidation catalyzed by

enzymes belonging to the cytochrome P450 family, especially CYP2D6, and susceptibility to cancer has aroused much interest. CYP2D6 enzyme participates in the activation to genotoxic intermediates, for example NNK (4-(methylnitrosamine)-1-(3-pyridil)-1-butanone), a procarcinogen contained in tobacco smoke, and deactivation of environmental xenobiotics such as carcinogens and toxic substances [11].

CYP2D6 enzyme also plays an important role in the metabolism of 20–25% of clinically important drugs, especially tricyclic antidepressants, selective serotonin reuptake inhibitors, neuroleptics, antiemetics, cardiological drugs and opioids [24, 35].

The CYP2D6 gene polymorphism, due to single nucleotide polymorphisms, gene duplication and deletion, is relatively well known. At least 60 CYP2D6 alleles are responsible for the about 200-fold variability in the metabolism. CYP2D6\*3 and CYP2D6\*4 are the most common nonfunctional CYP2D6 alleles in Caucasians population. The mutation in CYP2D6\*3 consists in a single base-pair deletion in exon 5 and in the case of CYP2D6\*4 in G to A transition in 1934 position what causes an altered reading frame and a premature stop codon. The carriers of deficient (CYP2D6\*3/\*4) or nonfunctional (CYP2D6\*4/\*4) alleles are poor metabolizers (PM). The homozygotes for wild-type CYP2D6 alleles (CYP2D6\*1/\*1) belong to extensive metabolizers (EM), while heterozygous persons to intermediate metabolizers (IM). Another relatively common phenomenon in humans is the duplication or amplification of CYP2D6. The mechanism involved in the gene duplication process is likely to be due to an unequal crossover between the two sister chromatids what causes deletion of the CYP2D6 gene (CYP2D6\*5) in one chromatid and duplication of the gene in the other one (CYP2D6\*x2). The presence of multiduplicated genes is attributable to additional unequal crossover events affecting chromatids that already contain duplicated genes. The carriers of three or more active alleles are ultra rapid metabolizers (UM). The clinical relevance of genetic polymorphism can be seen in the wide interindividual variability of therapeutic efficacy and in the spectrum of drug adverse effects [2, 9, 28].

The aim of this work was to evaluate whether patients with acute myeloblastic leukemia (AML) differ from healthy persons in their CYP2D6 genotype.

## Materials and Methods

The study was carried out in 34 patients with *de novo* acute myeloblastic leukemia before chemotherapy and 64 healthy persons as a control group. All subjects were of native Polish origin living in the southwestern (Lower Silesia) region of Poland. The healthy

**Tab. 1.** Data of patients with acute myeloblastic leukemia (AML) and healthy persons as a control group

Group	Number of persons	Age (years)				Gender (number of persons)	
		$\bar{x}$	$\pm$ SD	from	to	females	males
Patients with AML	34	55.7	12.7	20	79	18	16
Healthy persons	64	33.2	12.5	19	88	35	29

volunteers were members of Medical University staff and students. All subjects were nonpregnant unrelated persons with normal kidney and liver function.

The recruited patients comprised 18 women and 16 men between the ages of 20 and 79 years (mean age,  $55.7 \pm 12.7$ ). The control group included 35 females and 29 males, aged 19–88 years (mean age,  $33.2 \pm 12.5$ ). The data of patients with acute myeloblastic leukemia and healthy persons as a control group are shown in Table 1.

Informed consent was obtained in every case. The protocol for the study was approved by the Bioethics Committee of Wrocław Medical University.

Blood samples were collected in sterile glass tubes containing EDTA as an anticoagulant and stored as whole blood at  $-20^{\circ}\text{C}$  until analyzed. The genomic DNA was isolated from leukocytes of peripheral blood. Mutation at the CYP2D6\*3 and CYP2D6\*4 alleles was analyzed by polymerase chain reaction amplification and restriction fragment length polymorphism (PCR-RFLP) techniques based on the method described by Smith et al. [31]. Genotyping for the CYP2D6 gene amplification was performed by polymerase chain reaction amplification (PCR) techniques based on the method described by Lovlie et al. modified by Steijns et al. [20, 32]. Alleles carrying neither CYP2D6\*3, CYP2D6\*4 nor CYP2D6 gene amplification were classified as CYP2D6\*1 (wild-type) alleles.

The intergroup comparison was performed with the  $\chi^2$  test with the Yates' correction.

## Results

The CYP2D6 allele and genotype frequencies in the group of patients with AML and healthy subjects are

**Tab. 2.** CYP2D6 allele frequencies in the group of patients with acute myeloblastic leukemia (AML) and in healthy persons

CYP2D6 allele	Patients with AML		Healthy persons	
	n	frequency (%)	N	frequency (%)
*1	47	69.1	96	75.0
*3	1	1.5	2	1.5
*4	7	10.3 <sup>1</sup>	29	22.7
*1xn	12	17.6 <sup>2</sup>	1	0.8
*4xn	1	1.5	0	0.0
Total	68	100	128	100

<sup>1</sup>statistically significant difference in CYP2D6\*4 allele frequencies between group of patients with AML and healthy persons at  $p < 0.05$ ,  $\chi^2 = 3.74$ ; <sup>2</sup>statistically significant difference in CYP2D6\*1xn allele frequencies between group of patients with AML and healthy persons at  $p < 0.0001$ ,  $\chi^2 = 17.8$

summarized in Tables 2 and 3, respectively. The frequency of CYP2D6\*1, CYP2D6\*3, CYP2D6\*4, CYP2D6\*1xn, CYP2D6\*4xn alleles among 64 genotyped healthy persons was 75.0%, 1.5%, 22.7%, 0.8% and 0.0%, respectively. The group of 5 carriers of CYP2D6 deficient genes (7.8% in Polish population) consisted of only homozygous individuals carrying two nonfunctional alleles (CYP2D6\*4/\*4 genotype). In the control group, CYP2D6 gene amplification was detected in one person (1.6%).

**Tab. 3.** CYP2D6 genotypes frequencies in the group of patients with acute myeloblastic leukemia (AML) and healthy persons

CYP2D6 genotypes	Patients with AML		Healthy persons	
	n	frequency (%)	n	frequency (%)
*1/*1	16	47.1	37	57.8
*1/*1xn	10	29.4 <sup>3</sup>	1	1.6
*1/*3	1	2.9	2	3.1
*1/*4	4	11.8	19	29.7
*1xn/*4	2	5.9	0	0.0
*3/*4	0	0.0	0	0.0
*4/*4	0	0.0	5	7.8
*4/*4xn	1	2.9	0	0.0
Total	34	100	64	100

<sup>3</sup> statistically significant difference in CYP2D6\*1/\*1xn genotype frequencies between group of patients with AML and healthy persons at  $p < 0.0001$ ,  $\chi^2 = 14.6$

The frequency of CYP2D6\*1, CYP2D6\*3, CYP2D6\*4, CYP2D6\*1xn, CYP2D6\*4xn alleles among 34 genotyped patients with acute myeloblastic leukemia was 69.1%, 1.5%, 10.3%, 17.6% and 1.5%, respectively. The group of 13 carriers of CYP2D6 gene amplification (38.2%) consisted of 10 (29.4%) persons with one normal and one multiplied allele (CYP2D6\*1/\*1xn), 2 (5.9%) heterozygous individuals with one multiplied allele and one nonfunctional allele (CYP2D6\*1xn/\*4) and 1 (2.9%) homozygous person carrying multiplied nonfunctional alleles (CYP2D6\*4/\*4xn).

Most individuals, either in the group of patients or controls, had two active copies (CYP2D6\*1/\*1 or CYP2D6\*1xn/\*4), being classified as extensive metabolizers (EM). Heterozygous persons (CYP2D6\*1/\*3 or CYP2D6\*1/\*4) with one active and one deficient gene were predicted as intermediate metabolizers (IM), homozygous individuals carrying two nonfunctional alleles (CYP2D6\*4/\*4 or CYP2D6\*3/\*4 or CYP2D6\*4/\*4xn) as poor metabolizers (PM) and persons with CYP2D6 gene amplification (CYP2D6\*1/\*1xn) as ultra rapid metabolizers (UM) [38]. The expected frequencies of oxidation phenotype in patients with AML and healthy persons are summarized in Table 4.

Statistically significant difference were detected in gene amplification ( $p < 0.0001$ ,  $\chi^2 = 14.6$ ) and CYP2D6\*4 allele ( $p = 0.05$ ,  $\chi^2 = 3.74$ ) frequency be-

**Tab. 4.** The expected oxidation phenotype frequency in patients with AML and healthy persons

Expected oxidation phenotype	Patients with AML		Healthy persons	
	n	frequency (%)	N	frequency (%)
Ultra rapid metabolizers	10	29.4 <sup>4</sup>	1	1.6
Extensive metabolizers	18	53.0	37	57.8
Intermediate metabolizers	5	14.7	21	32.8
Poor metabolizers	1	2.9	5	7.8
Total	34	100	64	100

<sup>4</sup> statistically significant difference in ultra rapid metabolizer frequencies between group of patients with AML and healthy persons at  $p < 0.0001$ ,  $\chi^2 = 14.6$

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tween the group of patients with acute myeloblastic leukemia and healthy persons.

Our findings indicated an overrepresentation of UM and an underrepresentation of CYP2D6\*4 allele among patients with acute myeloblastic leukemia.

The odds ratio for ultra rapid metabolizers was statistically significantly about 25-fold greater in the group of patients with acute myeloblastic leukemia than in the group of healthy persons.

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## Discussion

The majority of chemical carcinogens, both exogenous (xenobiotics) and endogenous (e. g. steroid hormones), require biotransformation to activated forms to become carcinogenic. The CYPs are preeminent in catalyzing such bioactivations. The first association between CYP2D6 enzyme activity and the susceptibility to cancer (lung cancer) was described in 1984. Despite a large number of independent studies published since then, the role of CYP2D6 in cancer pathology remains still conflicting and inconclusive. For lung cancer more findings are controversial. It has been demonstrated by several independent groups that individuals carrying defective CYP2D6 gene show a small decrease and the carriers of duplicated active genes have increased risk of developing lung cancer, which rose parallelly with the number of active CYP2D6 genes. A substantial part of this controversy is due to the fact that CYP2D6 is likely to be only a minor factor in lung cancer, as compared for instance with the strong effect of smoking habits. Also individuals carrying two or more functional CYP2D6 genes have increased risk of developing liver and larynx cancer. Nevertheless, most of the reported studies lack an association of CYP2D6 genotype with head and neck cancer risk, and a few studies with positive findings only reported a minor association. In the case of melanoma, several small trials reported that individuals with defective genes were at increased risk. For breast, prostate, bladder, brain, renal, colorectal cancers, studies performed in several hundreds individuals reported an absence of relevant relations of malignant diseases with CYP2D6 genotype polymorphism. Finally, very limited or isolated studies indicated the absence of a major impact of CYP2D6 genotype on lymphomas, ovarian, anal, vulvar, pan-

creas, cervix and pituitary tumors [1–4, 8, 10, 11, 14, 22, 29, 33, 34, 36].

Before the development of genotyping procedures, the putative association of CYP polymorphisms and cancer risk was investigated by phenotyping analyses. The views concerning the relationship between genotype or phenotype and the risk of cancer development seem to be diversified. Some phenotyping studies provided evidence for a relationship between the ultra rapid sparteine oxidation phenotype and non-occupational bladder cancer susceptibility but further genotyping analyses failed to support such association [4, 8, 13, 25, 26]. These discrepancies may be caused by the allelic variants, that have been described recently, and too small number of participants in the genotyping studies, because several associations were reported just once, or discrepant findings do not permit to draw conclusions until more information is available. In some cases, the comparison of different studies is difficult since investigations were carried out in not numerous and non-homogenous groups of persons, by different genotyping techniques [1].

Evidence for polymorphism at CYP2D6 loci as a factor in cancer susceptibility has been reported for several cancers including hematological malignancies. To our knowledge, no studies have examined the ultra rapid metabolizer frequency in the group of patients with AML. Our findings indicated statistically significant overrepresentation of the CYP2D6 gene amplification and ultra rapid metabolizer among the group of patients with acute myeloblastic leukemia. These results comply with our earlier phenotyping study. In that research, the frequency of patients with metabolic ratio lesser than 1 was statistically significant greater in the group of patients with AML than in the control group [18].

Lemos et al. reported a significant association between the CYP2D6 extensive metabolizers genotype and leukemia [19]. However, Roddam et al. showed that inheritance of the CYP2D6 poor metabolizer phenotype was related with an increased risk of developing AML [27]. Most of the studies were done in the group of children with acute lymphoblastic leukemia or in patients with therapy or chemical-related acute lymphoblastic leukemia and indicated the absence of relevant association of ALL with CYP2D6 polymorphism [12, 15, 16, 21, 30]. Opposite findings presented in other studies may reflect geographical differences in the type of environmental carcinogens to which different populations are exposed.

To date, few studies have investigated the role of other CYPs in acute leukemia susceptibility. An association of CYP1A1\*2A [16, 17] or CYP1A1\*2B alleles [6] with increased risk to develop acute lymphoblastic leukemia in children was described, although negative findings in adult persons with AML or ALL have been reported. In the case of CYP2C19 carriers slow metabolizer CYP2C19 genotypes appear to be at increased risk to develop AML or ALL [27]. Negative reports of association of CYP3A4\*1B1 in children who developed therapy-related myeloid malignancies were published [5]. The CYP3A5\*3 variant allele was studied in 188 myeloid leukemia patients with negative findings [1]. The frequency of CYP3A5\*3 alleles in patients with therapy-related acute myeloid leukemia was studied in 279 children with acute lymphoblastic leukemia, and no association of CYP3A5\*3 alleles and increased risk of developing acute myeloid leukemia was observed [5], therefore, further studies are required to elucidate the relevance of such association.

Our study is the first trial which estimates the distribution of ultra rapid metabolizers in a population from Poland. The frequency of CYP2D6 gene amplification in Poland (1.6%) is in concordance with results obtained in other white populations in Northern and Central European countries. Its frequency in Caucasian individuals reaches 0.7% in Estonia, 1.2% in Finland and between 1.6%–1.9% in Germany [7, 37].

Also in our study, the frequencies of poor (7.8%) and intermediate (32.8%) metabolizers as determined by CYP2D6 genotype and distributions of CYP2D6\*1, CYP2D6\*3 and CYP2D6\*4 alleles (75.0%, 1.5%, 22.7%, respectively) are similar to those in Polish and other white population [7, 23, 37].

CYPs are also prominent players in the biotransformation of cytostatics, enhancing or diminishing the efficacy of the anticancer chemotherapy. Several anti-neoplastic drugs, e.g. tamoxifen, its 4-hydroxy and 3-hydroxy metabolites, taumostine and other drugs frequently used in cancer patients as antiemetics or analgesic are metabolized by CYP2D6 [11].

In conclusion, it should be stated that our results represent some evidence for a possible relationship between ultra rapid metabolizer genotype and the higher susceptibility to development of acute myeloblastic leukemia. They suggest that about 30% of these patients may require higher doses or shorter dosing intervals than those usually applied to reach a therapeutic effect [2].

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