

CYP1B1 gene polymorphism modifies pancreatic cancer risk but not survival

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Pancreatic cancer represents one of the biggest problems of current oncology. The risk factors of pancreatic cancer development, as well as factors affecting survival are poorly understood. Since biotransformation enzymes modify detoxification of carcinogens, we supposed, that a relationship between their polymorphism and the risk of pancreatic cancer development and eventually its clinical outcome may exist.

Associations of so far not studied cytochrome P450 1B1 (CYP1B1) polymorphisms with pancreatic cancer risk were investigated by case-control study. A total of 754 participants were recruited during study period. All patients were followed to determine their treatment and overall survival.

Carriers of rare genotype Val/Val in codon 432 of *CYP1B1* (rs1056836) were under significantly lower risk of pancreatic cancer than wild type carriers ($p=0.035$). Carriers of heterozygous genotype ($p=0.033$) and rare allele Val ($p=0.015$) were also under lower risk than wild type carriers. When histology-verified patients were analyzed separately, even more significant associations were found ($p=0.016$, $p=0.009$, $p=0.003$, respectively). On the contrary, *CYP1B1* polymorphism in codon 453 (rs1800440) did not significantly associate with pancreatic cancer risk. Median survival of patients with rare homozygous genotype Val/Val in *CYP1B1*-codon 432 was longer but not significantly different from those with wild-type homozygotes. The same was true for *CYP1B1*-codon 453 wild-type homozygotes in comparison with Ser/Ser rare homozygotes.

CYP1B1 polymorphism in codon 432 seems to modify the risk of pancreatic cancer development and should be further studied.

Keywords. Pancreatic cancer, CYP1B1, polymorphism, risk, survival

Pancreatic cancer represents one of the biggest challenges of current oncology due to its poor prognosis. The overall incidence of pancreatic cancer is growing rapidly. It has risen from 6.3 per 100,000 residents in 1980 to 9.6 per 100,000 in 2005 [1]. In the Czech Republic, pancreatic cancer is the eighth most prevalent cancer with almost 1200 new cases diagnosed annually. Despite a high mortality rate linked to pancreatic cancer, there is only little knowledge about its etiology. Therefore, it is important to understand how genetic factors contribute to clinical outcome of this disease.

Cytochrome P450 (CYP, EC 1.14.14.1) enzymes catalyzes a large number of reactions modifying dietary and smoking-derived pro-carcinogens including polycyclic aromatic hydrocarbons (PAHs), heterocyclic and aryl amines, and nitroaromatic hydrocarbons [2, 3]. When activated, these pro-carcinogens produce reactive intermediates that can cause

DNA damage and promote carcinogenesis. therefore this role, CYP1B1 (OMIM: 601771) is extensively investigated as a potential risk factor in human cancer [4]. Genetic polymorphism in *CYP1B1* was recently associated with increased risk and clinical outcome of wide variety of human cancers with suspected environmental component including colorectal [5], lung [6], prostate [7], renal cell [8], head and neck [9] and pancreatic [10] cancers.

CYP1B1 is located on chromosomal region 2p21–22 and consists of two introns and three exons of which two are translated into protein. A number of single nucleotide polymorphisms were described in *CYP1B1* [11]. We have focused on two most studied missense *CYP1B1* polymorphisms in codons 432 (rs1056836) and 453 (rs1800440) localized in exon 3 and their role in pancreatic cancer risk. These polymorphisms are associated with amino acid substitutions, Val432Leu and

Asn453Ser in the heme-binding domain of the enzyme. Therefore these mutations may interfere with heme incorporation, by affecting the hinge region and/or the conserved core structures (CCS) that determine the proper folding and heme-binding ability of P450 molecules [12]. Thereby these mutations of *CYP1B1* cause some alterations in substrate specificity and catalytic activity. Several studies indicate that these polymorphic variants of *CYP1B1* have greater hydroxylation activities and are considered to be candidates for cancer susceptibility [13, 14]. It has been reported that Leu allele carriers in the *CYP1B1* codon 432 are more active in oxidation of benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol (in the presence of epoxide hydrolase) than the Val allele carriers which further lead to formation of carcinogen benzo[*a*]pyrene-7,8-diol-epoxide [15]. Since these polymorphisms may also influence the biotransformation of anticancer drugs, we investigated whether a relationship between polymorphisms and the clinical outcome (assessed by analysis of overall survival) exists.

Materials and methods

Study subjects. The association between pancreatic cancer risk and genetic polymorphisms was investigated in a case-control study. The enrollment of subjects to the study started in September 2004 and was closed in February 2008. A total of 754 participants were included into the study. Patients with pancreatic cancer were recruited at five oncology centers located in Prague, Pribram, Liberec, Rakovnik and Zlin. Patients were eligible for the study, when they fulfilled at least one of the following criteria:

- patient had histology- or cytology-confirmed pancreatic adenocarcinoma or
- patient had at least three of the following clinical signs of pancreatic cancer (weight loss, anorexia/cachexia, obstructive jaundice, mass on CT / MRI / endoscopic ultrasound scans, tumor markers elevation).

Controls were selected to have similar gender and age distribution as cases. We used two different control groups to increase the power of study. The first control group was composed of healthy volunteers recruited by general practitioners during regular preventive checkups. Second group was composed of blood donors. The first primary endpoint was the association between the risk of pancreatic cancer and *CYP1B1* polymorphisms, the second was the overall survival (OS), defined as the interval between the date of first histological verification of pancreatic cancer until death from any cause. Patients were followed through October 30, 2008. We performed a review of medical records to obtain information on chemotherapy and/or radiation for all eligible patients. Since the patient enrollment covered almost all regions of Czech Republic, we consider this study being adequately representative for Czech population. The design of the study was approved by the Ethical Committee of the 1st Medical Faculty, Charles University in Prague, Czech Republic.

Genotyping. Blood was collected during diagnostic procedures using tubes with K₃EDTA anticoagulant. DNA was isolated from lymphocytes using the phenol/chloroform extraction method [16]. Polymorphisms in *CYP1B1* were assayed using allelic discrimination with TaqMan Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA) by real time PCR in RotorGene 6000 (Corbett Research, Brisbane, Australia). The respected polymorphisms and assays were, *CYP1B1* codon 432 (Leu432Val, rs1056836 assay no.: C_3099976_30) and codon 453 (Asn453Ser, rs1800440, C_11642651_30). Determination was performed according to instructions of manufacturer (Applied Biosystems). Quality control was performed by reanalysis of 10% of randomly selected samples. Results were 100% concordant. Oligonucleotide primers were synthesized by Generi Biotech (Hradec Kralove, Czech Republic).

Statistical analyses. Statistical analyses were processed by the statistical software CRAN 2.4.0. The mean, median, SD, variance, minimum, maximum, quartiles, frequencies and other basic statistical measurements were computed in given groups and subgroups. The overall survival of given groups and subgroups was determined using Kaplan-Meier's survival distribution functions. The Log-rank test was used for evaluation of different survivals among investigated groups and subgroups. For determination the risk factors in relation to overall survival Hazard Ratio was computed by the Cox proportional hazard model. Odds ratios (OR) and confidence intervals for examining the association between genetic factors and cancer risk were estimated by logistic regression.

Results

General characteristics of participants. 285 cases and 469 controls entered into the study. Among pancreatic cancer patients, there were 132 patients with histology-verified diagnosis. In 115 patients, the diagnosis was based on clinical symptoms. 38 patients were excluded from the study due to other than pancreatic cancer diagnosis (review process found 15 individuals with pancreatitis and 23 with other diagnosis). Randomly selected controls were healthy individuals and consisted of two independent groups: 179 healthy subjects recruited by general practitioners in Prague during the 3rd month after the cases recruitment, and 290 blood donors recruited from two centers in Prague and Pribram. Cases comprised of 39.6% females and 60.4% males whereas controls included either 46.4% females and 55.6% males (GP group) or 31.9% females and 68.1% males (BTS group). The difference in sex distribution between cases control groups was not statistically significant. The average age of cases was 61.4 ± 10.8 years vs. 59.2 ± 12.0 years in GP group and 40.0 ± 11.8 years in BTS group.

Clinical characteristics of the patients. The first manifestation of the disease was obstructive icterus in almost 70 % of patients whereas the rest usually reported pain and weight loss. About 34% of histology-verified cases underwent surgery

Table 1: Association of CYP1B1 polymorphisms with pancreatic cancer risk

	Cases, N (%)		Controls, N (%)	OR (95% CI) ^a	
	All	Histology-verified		All cases	Histology-verified
CYP1B1-432					
Leu/ Leu	91 (36.8)	55 (41.7)	131 (28.0)	reference	reference
Leu/Val	124 (50.2)	62 (47.0)	259 (55.3)	0.69 (0.49-0.97)	0.57 (0.38-0.87)
Val/Val	32 (13.0)	15 (11.3)	78 (16.7)	0.59 (0.36-0.96)	0.46 (0.24-0.86)
Leu/Val+	156	77	337	0.67 (0.48-0.97)	0.54 (0.36-0.81)
Val/Val					
qVal^b			0.44		
CYP1B1-453					
Asn/Asn	172 (69.6)	87 (66.0)	326 (69.5)	reference	reference
Asn/Ser	63 (25.5)	39 (28.5)	126 (26.9)	0.95 (0.67-1.35))	1.19 (0.75-1.79)
Ser/Ser	12 (4.9)	6 (4.5)	17 (3.6)	1.35 (0.63-2.86)	1.32 (0.51-3.45)
Asn/Ser+	75	45	143	0.99 (0.71-1.39))	1.18 (0.78-1.78)
Ser/Ser					
qSer^b			0.17		

^a OR=odds ratio, 95% CI=95% confidence interval

^b frequency of the rare allele in control group

of which about 50 % was radical surgery. The first palliative chemotherapy was predominantly gemcitabine. 5-Fluorouracil was used in the rest of anticancer therapy-treated patients. Due to the low performance status only 3 patients received II. line of palliative treatment (capecitabine or 5-fluorouracil).

Polymorphisms and pancreatic cancer risk. There were no significant differences in CYP1B1 rare allele frequencies and genotype distributions between GP and BTS control groups allowing us to pool these control groups for further analyses. Evaluation of genotype distribution and allele frequencies in cases and controls showed that carriers of rare genotype Val/Val in codon 432 of CYP1B1 were under significantly lower risk of pancreatic cancer than wild type carriers (Table 1, p=0.035). Carriers of heterozygous genotype (p=0.033) and rare allele Val (p=0.015) were also under lower risk than wild type carriers. The same was true for histology-verified patients when analyzed separately (p=0.016 for rare genotype, p=0.009 for heterozygotes, and p=0.003 for rare allele carriers vs. wild type carriers). On the contrary, CYP1B1 polymorphism in codon 453 did not significantly associate with pancreatic cancer risk (Table 1). There were not enough participants for analysis of combined effect of both CYP1B1 polymorphisms.

Overall survival. CYP1B1 polymorphisms did not significantly modify overall survival of either all pancreatic cancer patients or histology-verified subgroup of patients. Median survival of patients with rare genotype Val/Val in codon 432 of CYP1B1 was 1.73 year (95 % CI=0.66-1.34), with heterozygous genotype Val/Leu was 0.91 year (95 % CI=0.76-1.42) in comparison with wild-type Leu/Leu carriers (1.12 year 95 % CI=0.90-1.37). Median survival of patients with rare genotype Ser/Ser in codon 453 of CYP1B1 was 0.95 year (95 % CI=0.31-0.97), with heterozygous genotype Asn/Ser was

0.87 year (95 % CI=0.63-2.10) in comparison with wild-type Asn/Asn carriers (1.17 year; 95% CI=0.91-1.37). Median survival of histology-verified patients with CYP1B1-codon 432 genotypes Val/Val, Leu/Val, and Leu/Leu was 1.78 year 95 % CI=0.34-2.49), 0.87 year (95 % CI=0.58-1.42), and 1.12 year (95 % CI=0.85-2.65), respectively. Median survival of histology-verified patients with CYP1B1-codon 453 genotypes Ser/Ser, Asn/Ser, and Asn/Asn was 0.42 year (95 % CI=0.05-0.93), 0.87 year (95 % CI=0.48-1.77), and 1.17 year (95% CI=0.85-1.55), respectively.

Discussion

The risk factors leading to the pancreatic cancer development are poorly understood. Minority of these cancers can be linked to currently known hereditary cancer syndromes like syndrome of hereditary pancreatitis or hereditary breast-ovarian carcinomas, but generally no explanation for the majority of pancreatic carcinomas exists. Previous studies suggested that higher risk of pancreatic cancer may be associated with certain polymorphisms in metabolizing genes including CYPs. However, virtually no study was performed specifically on population of Czech origin or other Slavic ones. We focused our attention on two CYP1B1 polymorphisms frequently studied in sporadic cancers other than pancreatic.

In our study, a significant association between CYP1B1 polymorphism in codon 432 and the pancreatic cancer risk was observed. Histology-verified cases showed even more significant trend in the same direction, i.e. higher risk in carriers of wild type genotype in comparison with rare allele carriers (p=0.003). Recently, higher levels of 4-aminobiphenyl-hemoglobin adducts were observed in wild Leu allele carriers

in the *CYP1B1* codon 432 as compared to the rare genotype. A significant interaction between these *CYP1B1* genotypes and the level of exposure was found as well ($p=0.003$, ref. 17). Thus, the wild type allele which is more active than the rare one [18] may contribute to enhanced exposure-related damage of biomacromolecules and subsequently to carcinogenesis.

No association of the second studied polymorphisms in codon 453 with the risk was found in our study. The frequencies of rare *CYP1B1* alleles in our pooled control group ($n=469$) were similar to those published in other Caucasian populations (codon 432 – 0.45; ref. 19 and codon 453 – 0.18; ref. 5). Case-control study on role of *CYP1B1* polymorphisms in pancreatic cancer risk was not published so far. However, there were published studies on polymorphisms in other CYPs and metabolizing genes. Lee et al. [20] did not find any significant association of *CYP1A1*, *CYP2D6*, and *CYP2E1* haplotypes with pancreatic cancer risk in a small case-control study on Korean population. In contrast, another study reported that polymorphisms in *CYP1A2* and *NAT1* genes modify the risk of pancreatic cancer [21]. Moreover, a significant interaction between *NAT1* genotype and dietary mutagen intake modifying the risk of pancreatic cancer was observed among men but not women and suggested the existence of gender-specific susceptibility to dietary mutagen exposure [22]. Thus, polymorphisms in metabolic genes may modulate pancreatic cancer risk and present interesting topic for further studies.

We also examined the influence of both *CYP1B1* polymorphisms on the overall survival of the disease. Although non-significant, a trend towards longer survival of patients with rare genotype Val/Val in codon 432 of *CYP1B1* in comparison with patients carrying wild-type alleles was observed. There is lack of data in the literature to corroborate this result more thoroughly. Thus, due to the poor prognosis of pancreatic cancer patients and the frequent resistance of the disease to standard anticancer therapy, it seems that *CYP1B1* polymorphisms most probably lack prognostic significance.

In conclusion, the *CYP1B1* polymorphism in codon 432 seems to influence pancreatic cancer risk but not prognosis in the Czech population. As the data on genetic background of pancreatic cancer are inconsistent worldwide, further research is needed to find factors contributing to pancreatic cancer development and progression.

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