Variant alleles of the CYP1B1 gene are associated with colorectal cancer susceptibility

Joanna Trubicka1, Ewa Grabowska-Kłuszo1, Janina Suchy1, Bartłomiej Masoje1, Pablo Serrano-Fernandez1, Grzegorz Kurzawski1, Cezary Cybulski1, Bohdan Górski1, Tomasz Huzarski1, Tomasz Byrski1, Jacek Gronwald1, Elżbieta Złowocka1, Józef Kladny2, Zbigniew Banaszkiewicz3, Rafał Wiśniowski4, Elżbieta Kowalska1, Jan Lubinski1, Rodney J Scott5,6*

Abstract

Background: CYP1B1 is a P450 enzyme which is involved in the activation of pro-carcinogens to carcinogens as well as sex hormone metabolism. Because differences in the activity of the enzyme have been correlated with variant alleles of single nucleotide polymorphisms (SNPs), it represents an attractive candidate gene for studies into colorectal cancer susceptibility.

Methods: We genotyped 597 cancer patients and 597 controls for three CYP1B1 SNPs, which have previously been shown to be associated with altered enzymatic activity. Using the three SNPs, eight different haplotypes were constructed. The haplotype frequencies were estimated in cases and controls and then compared. The odds ratio for each tumour type, associated with each haplotype was estimated, with reference to the most common haplotype observed in the controls.

Results: The three SNPs rs10012, rs1056827 and rs1056836 alone did not provide any significant evidence of association with colorectal cancer risk. Haplotypes of rs1056827 and rs10012 or rs1056827 and rs1056836 revealed an association with colorectal cancer which was significantly stronger in the homozygous carriers. One haplotype was underrepresented in the colorectal cancer patient group compared to the control population suggesting a protective effect.

Conclusion: Genetic variants within the CYP1B1 that are associated with altered function appear to influence susceptibility to a colorectal cancer in Poland. Three haplotypes were associated with altered cancer risk; one conferred protection and two were associated with an increased risk of disease. These observations should be confirmed in other populations.

Background

Adverse interactions between DNA and environmental toxins may be mitigated through a range of biologic defense mechanisms, including DNA repair, cell cycle checkpoint control and xenobiotic clearance enzymes. Xenobiotic clearance is important for the removal of carcinogens and is primarily accomplished by hydroxyl conjugation, involving enzymes in the cytochrome P450 pathway [1]. Cancers of the colon, lung, larynx, kidney and pancreas have been shown to be associated with environmental exposures to various carcinogens [2,3] and polymorphisms in several key enzymes involved in xenobiotic clearance have been linked to the risks of various cancers. One enzyme of particular importance is CYP1B1, which is encoded by a polymorphic gene [3]. A number of polymorphisms in this gene have been shown to affect the activity of the encoded protein [4,5]. Four polymorphisms, occurring at codons rs10012, rs1056827, rs1056836 and rs1800440, all of which result in single amino acid substitutions that result in an altered enzyme activity are of particular interest [5]. CYP1B1 is primarily involved in the hydroxylation of 17β-estradiol at the 2-OH and 4-OH positions, which can then be oxidized to semiquinones and quinines [1].

* Correspondence: rodney.scott@newcastle.edu.au

© 2010 Trubicka et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Both semiquinones and quinones are electrophilic metabolites that can form DNA adducts thereby potentially introducing mutations into the genome [6]. Generation of 2-OH catechol estrogens does not appear to result in deleterious affects whereas the 4-OH catechol derivative has been shown to be associated with an increase in DNA single strand breaks as well as increased nuclear levels of 8-hydroxyguanosine [5,7,8]. Both of these undesirable outcomes alter the risk of malignancy by increasing the likelihood of introducing mutations into the genome and consequently the risk of malignancy.

As part of a major initiative to identify major cancer susceptibility genes for colorectal cancer, we investigated three common variants in the CYP1B1 gene that individually confer a difference in the activity of the encoded protein. All three variants, codon 48G > C (rs10012), codon 119G > T (rs1056827) and 432 G > C (rs1056836) have been shown to result in an increased metabolism of 16α-OH-estradiol [5]. An investigation of three of the CYP1B1 SNP polymorphisms was undertaken to determine whether, alone or in combination, these SNPs were associated with an altered likelihood of disease. A fourth CYP1B1 variant, SNP 453A > G (rs1800440) was not investigated in the present study because it does not occur at sufficiently high frequency to allow a meaningful analysis.

**Methods**

**Study subjects**

The Polish population is extremely homogeneous and lends itself to population based studies as there are no significant ethnic differences to take into consideration. 597 colorectal cancer patients were enrolled in the study. Enrollment occurred between 1998 and 2006 from 3 cities in Poland (Table 1). Patients were asked to participate during a visit to the outpatient oncology departments of the various participating hospitals affiliated with the Hereditary Cancer Center in Szczecin. To estimate the prevalence of the various CYP1B1 genotypes and haplotypes in the underlying Polish population, 597 sex and age-matched controls were invited to participate in the study all of whom resided in the city and surrounds of Szczecin.

The study protocol was approved by the ethics committee of the Pomeranian Medical University and each participant signed a consent form for the study prior to enrolment.

**DNA isolation**

DNA was extracted from peripheral blood lymphocytes of individuals using the non-enzymatic, rapid method without modification [9].

**Polymorphism Analysis**

The A119 S variant was identified by restriction fragment length polymorphism PCR using CTCGTTCGCT CGCCCTGGCGC and GAAGTTGCGCATCATGCTGT primers. The PCR reactions was carried out in PTC-200 Peltier DNA Thermal Cycler MJ Research. Detailed experimental PCR conditions are available on request. Amplified DNA was digested with Eam 1105I enzyme and size separated on a 3% agarose gel. The PCR product length, before and after digestion were 136 base pairs (bp) and 114 bp, respectively (see Fig 1) All positive results were verified by a reverse RFLP-PCR using a Pdil enzyme (Fermentas, St Leon Rot, Germany). Finally, a random selection of cases harbouring the GG, GT or TT genotypes were subjected to direct sequencing using the BigDye Terminator Ready Reaction Kit v3.0 (Applied Biosystems, Foster City, CA) and analyzed in an ABI PRISM 377 DNA sequencer (data not shown).

A similar protocol was performed for the 142G > C and 432C > G change using the primer pairs TCCATCCAGCAGACCACAGCT and GCCGGACACCACACTGGGAAG and ATGGGTTCTCCAGCTTTGT and TATGGAGCACACCTCACCTG, respectively. The 142G > C polymorphism was identified using the restriction enzyme AvaI enzyme (Fermentas). The resultant products were size separated on 4% agarose gels. The uncut product was 335 bp in size and the cut product was predicted to yield 4 fragments of 230, 105 and 91 base pairs. Only the 230, 105 and 91 base pair

---

Table 1 Sites and period of specimen collection for the patients samples

| Time period of collection of cases | 1998-2006 |
| Hospitals where patients were collected | Bielsko-Biała, Bydgoszcz, Szczecin |
| Age range of diagnosis | 21-92 |

---

Figure 1 Representative gel of RFLP-PCR for change 355G > T (A119S). Lanes: K1 positive control homozygote 355TT, K2 positive control heterozygote 355GT, K3 positive homozygote 355GG, 1+ homozygote 355TT, lanes 2, 3, 7 & 8 heterozygote 355 GT, lanes 4-6 & 9 homozygote 355 GG, K0 negative control, MD pUC Mix 8
fragments were visualized, since the 14 base pair product was too small to be readily detected using this approach (see Fig 2). In addition, randomly selected cases with G/G, C/C and C/G variants were sequenced (as described above) in order to confirm the presence of the R48G change (data not shown). The 432C > G change was identified using a similar protocol but the enzyme used for the RFLP was the OliI restriction endonuclease (Fermentas, St Leon Rot, Germany) which resulted in two fragments of 132 bp and 491 bp in size in samples that contained the C nucleotide (see Fig 3). Randomly selected cases with G/G, C/C and C/G variants were sequenced in order to confirm the presence of the 432G > C change.

**Statistical analysis**

The frequencies of the individual genotypes were compared between the cases and controls. Statistical significance of the odds ratios were tested using the chi-squared analysis (with Yates correction for continuity) and Fisher’s exact test, where appropriate. Bonferroni correction was applied to take into account multiple testing.

Eight haplotypes could be generated from the three SNPs. The frequencies of each of these were estimated in the cases and controls using the genetic statistical package haplo.stats for R http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html as reported by Schaid et al. [10]. The CGG haplotype was the most common and was used as the standard by which the odds ratios were calculated. The association between the presence of a particular haplotype and cancer risk was determined by the haplo.stats software using a score test (haplo.score) included in the package which is designed to detect statistically significant differences in the distribution of the estimated haplotype frequencies. Each of the three SNPs was in Hardy-Weinberg equilibrium in the cases and controls.

Each SNP of the CYP1B1 gene generated three genotypes, including two homozygote states and one heterozygote state. For each SNP, for each site, the odds ratio and 95% confidence intervals were constructed. For these comparisons, the most common allele was considered to be the normal allele and odds ratios were constructed with reference to individuals with two common alleles. The control population for this study was used to determine the relative frequency of the CYP1B1 alleles in the underlying Polish population.

**Results**

For each individual SNP genotyped no significant association between it and colorectal cancer was observed (see Table 2) suggesting that disease risk was not influenced by any of the SNPs alone. This suggests that the resultant affect of each of the polymorphisms alone on protein function was, at best, weak. Since each SNP chosen for this investigation has previously been associated with a change of function in the CYP1B1 gene, a genotype analysis was undertaken comparing two SNPs that were in proximity to one another to determine if the combination of two SNPs was more likely to be associated with disease risk. This analysis entailed assessing

---

**Table 2 Odds ratios for colorectal cancer associated with the three CYP1B1 genotypes**

| SNP      | CASES | CONTROLS | OR | P-VALUE | 95% CI |
|----------|-------|----------|----|---------|-------|
| rs10012  | CC    | 261      | 265| 0.9     | 0.9-1.1|
|          | GC    | 266      | 265| 1.0     | 0.8-1.2|
|          | GG    | 70       | 67 | 1.0     | 0.8-1.5|
| rs1056827| GG    | 249      | 277| 0.8     | 0.6-1.0|
|          | TG    | 271      | 259| 1.0     | 0.9-1.4|
|          | TT    | 77       | 61 | 1.3     | 0.9-1.8|
| rs1056836| CC    | 214      | 206| 1.0     | 0.8-1.3|
|          | CG    | 275      | 265| 1.0     | 0.8-1.3|
|          | GG    | 108      | 127| 0.8     | 0.6-1.0|

---

Figure 2: Representative gel of RFLP-PCR for 142C > G (R48G).

Lanes: KO negative control, K+ positive control (heterozygote 142CG), MD pUC Mix 8, 1 homozygote 142CC, 2 homozygote 142CC, 3 homozygote 142CC, 4 homozygote 142CG, 5 homozygote 142CG, 6 heterozygote 142CG, 7 heterozygote 142CG.

Figure 3: Representative gel of RFLP-PCR for change CYP1B1-V432L (4329G > C).

Lanes MD pUC Mix 8, K+ positive control heterozygote 4329 GC (V432L), KO negative control. Lanes 1, 2, 4-6 heterozygote 4329GC, lane 3 homozygote 4329GG, lane 8 homozygote 4329CC.
rs1056827 with rs10012 and rs1056827 with rs1056836 to determine if these combinations were associated with CRC risk. The most significant finding of this analysis was the identification of three genotypes, two of which were associated with an increased risk of disease and one that was protective (see Table 3). The combination of homozygous wild type rs10012 and heterozygous rs1056827 was associated with a significant increase in CRC risk OR 2.4 CI 1.41-4.05; P 0.001. When rs1056827 SNP was present in the homozygous state an even greater effect was observed (OR 7.1 CI 1.61-31.58; P = 0.04).

When rs1056827 was investigated with rs1056836 a similar trend was forthcoming (Table 4), where homozygous carriers of the rs1056827 SNP were more likely to develop colorectal cancer compared to their wild type counterparts OR 10.2 CI 1.295-79.61; P = 0.09, after Bonferroni correction) in the presence of homozygous carriers of the rs1056836 SNP. In support of this observation, a similar trend for heterozygote carriers of the rs1056836 allele was also observed in the colorectal cancer group (OR 6.1 CI 1.36-27.39; P = 0.12, after Bonferroni correction). Interestingly, individuals who were heterozygous for rs1056836 and 1056827 but wild type for rs10012 were not associated with an increased risk of disease.

In order to determine if there were groups of individuals who may be greater risk by being homozygous for all three SNPs we investigated the combination of homozygote carriers against each other, where the wild type haplotypes were considered to represent population risk (see Table 5). This analysis was restricted as a result of there being too few homozygote carriers available to undertake an exhaustive analysis of disease risk conferred by all possible combinations of genotypes. Notwithstanding, we were able to examine enough patients and controls to determine that homozygous for the following haplotype C-T-G appeared to be significantly at risk of developing colorectal cancer (OR 21.4 CI 1.2-365.56; P = 0.0019). The combination of being homozygote for rs1056827 and rs1056836 SNPs did not appear to be at any increase risk of disease.

### Table 3 Analysis of genotypes R48G and A119 S and frequency in colorectal cancer

| CYP1B1 | CASES n = 597 | CONTROL n = 597 | STATISTICAL ANALYSIS |
|--------|--------------|----------------|---------------------|
| rs10012 | rs1056827    | n Frequency [%] | n Frequency [%] OR p-value |
| GG     | 199          | 33.3           | 241                | 40.4 | 0.7 | 0.08* |
| CC     | 48           | 8.0            | 21                 | 3.5  | 2.4 | 0.001* |
| TT     | 14           | 2.4            | 2                  | 0.3  | 7.1 | 0.004* |
| GG     | 38           | 6.4            | 29                 | 4.9  | 1.3 | 0.3 |
| CG     | 216          | 36.2           | 233                | 39.0 | 0.9 | 0.3 |
| TT     | 12           | 2.0            | 4                  | 0.7  | 3.0 | 0.1 |
| GG     | 12           | 2.0            | 6                  | 1.0  | 2.0 | 0.2 |
| CG     | 51           | 8.54           | 55                 | 9.2  | 0.9 | 0.7 |

* after Bonferroni correction

### Table 4 Analysis of genotypes A119 S and V432L and frequency in colorectal cancer

| CYP1B1 | CASES n = 597 | CONTROL n = 597 | STATISTICAL ANALYSIS |
|--------|--------------|----------------|---------------------|
| rs1056827 | rs1056836 | n Frequency [%] | n Frequency [%] OR p-value |
| GC     | 103          | 17.2           | 119                | 20.0 | 0.8 | 0.3 |
| GG     | 86           | 14.4           | 112                | 18.8 | 0.7 | 0.1 |
| GC     | 160          | 26.8           | 143                | 24.0 | 1.2 | 0.3 |
| GG     | 12           | 2.0            | 14                 | 2.4  | 0.9 | 0.8 |
| GC     | 55           | 9.2            | 58                 | 9.7  | 0.9 | 0.8 |
| GG     | 10           | 0.8            | 1                  | 0.2  | 10.2 | 0.09^ |

# before Bonferroni correction 0.015
^ before Bonferroni correction 0.011
Table 5 Haplotype analysis of CYP1B1 and colorectal cancer risk

| CYP1B1 | rs10012 | rs1056827 | rs1056836 | ALL | OR | p-value |
|--------|---------|-----------|-----------|-----|----|---------|
| CC     | GG      | CC        | 1.0       | 1.0 |    |         |
|        |         | GG        | 0.7       | 0.1 |    |         |
| TT     | CC      | 0.2       | 0.5       |     |    |         |
|        | GG      | 21.4      | 0.00019*  |     |    |         |
| GG     | GG      | CC        | 3.0       | 0.2 |    |         |
|        |         | GG        | 7.0       | 0.3 |    |         |
| TT     | CC      | 0.9       | 0.7       |     |    |         |
|        | GG      | 0.3       | 1.0       |     |    |         |

*After Bonferroni correction.

Discussion
This study examines the potential influence of CYP1B1 genetic variants alone, and in combination, on colorectal cancer risk. Each of the three SNPs when considered individually appeared not to be associated with disease risk for colorectal cancer. When considered together, the magnitude of the colorectal cancer risk appeared to be consistent yet modest. One previous report of CYP1B1 and CRC risk provided some evidence for an association with disease but this disappeared once a correction was applied for multiple testing [11]. The report also concluded even though no unequivocal findings were identified the role of CYP1B1 in colorectal cancer risk could not be ruled out, especially in light of histopathological evidence indicating that CYP1B1 is overexpressed in colorectal cancers [12].

Several studies have examined the relationship between individual CYP1B1 polymorphisms and cancer risk [12-17]. The strength of the current study is the investigation of three CYP1B1 SNPs in an ethnically-homogeneous population of controls and patients with one of the most frequently observed malignancies in Poland. The effects of the three SNPs appear modest and would not have been identified in smaller studies.

The variants in codons 119 and 432 are associated with different substrate specificities and consequently the catalytic activity of the enzyme [16,17]. There is evidence that the CYP1B1 variants R48G, A119 S and L432V all exhibit greater catalytic 4-hydroxylation activity than the wild type enzyme [8], which may provide a functional clue as to why there appears to be an, albeit small, association with disease risk.

Finally, in studies similar to the one reported herein it would have been improved by including the effects of environmental factors on disease risk. The current study was not intended to address environmental influences on disease risk rather to determine whether or not there is an over-representation of genetic markers in a case compared to a control population thereby providing information on the relative involvement of the genetic marker on disease risk.

In summary, the CYP1B1 variants examined in this study suggest that they contribute to inter-individual differences in cancer risk and are potentially valuable in genetic risk assessment. These findings have potentially important implications for genetic risk assessment and for prevention studies, but it is important that they be confirmed in other colorectal cancer populations prior to introducing them in the clinical setting.

Conclusion
Genetic variants within the CYP1B1 that are associated with altered function appear to influence susceptibility to a colorectal cancer in Poland. Three haplotypes were associated with altered cancer risk; one conferred protection and two were associated with an increased risk of disease. These observations should be confirmed in other populations.

Acknowledgements
This study was funded from the Pomeranian Medical University. The authors would like to thank the participants for their contribution to this study.

Author details
1Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland. 2Department of Surgery, Pomeranian Academy of Medicine, Szczecin, Poland. 3Department of Surgery, Medical Academy, Bydgoszcz, Poland. 4Regional Oncology Hospital, Bydgoszcz, Poland. 5Department of Medical Genetics, School of Biomedical Sciences, Faculty of Health, University of Newcastle and the Hunter Medical Research Institute, Newcastle NSW Australia. 6Children's Cancer Research Group, Hunter Medical Research Institute, John Hunter Hospital, Lookout Road, New Lambton, NSW 2305 Australia.

Authors’ contributions
JT participated in the design and coordination of the study and carried out the molecular genetic studies, performed the statistical analysis and the manuscript. EGK participated in the collection of samples and carried out the molecular genetic studies. JS participated in the collection of samples. BM performed the statistical analysis. PSF participated in the design of the study. CC participated in the design of the study. BG participated in the collection of samples. TB participated in the collection of samples. EZ carried out the molecular genetic studies. JK participated in the collection of samples. TH participated in the collection of samples. JK participated in the collection of samples. JR participated in the collection of samples. EGK participated in the collection of samples and carried out the molecular genetic studies. JS participated in the collection of samples.

Competing interests
The authors declare that there are no competing interests.

Received: 29 August 2009 Accepted: 11 August 2010 Published: 11 August 2010

References
1. Spink DC, Hayes CL, Young NR, et al: The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17 beta-estradiol 4-hydroxylase. J Steroid Biochem Mol Biol 1994, 51:251-8.
2. Landi MT, Bergen AW, Baccarelli A, et al.: CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. Toxicology 2005, 207:191-202.

3. Agundez JA: Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 2004, 5:211-24.

4. Bailey LR, Roodi N, Dupont WD, Parl FF, et al.: Association of cytochrome P450 1B1 (CYP1B1) polymorphism with steroid receptor status in breast cancer. Cancer Res 1998, 58:5038-41.

5. Shimada T, Watanabe J, Kawajiri K, et al.: Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999, 20:1607-13.

6. Chakravarti D, Mailander PC, Li KM, et al.: Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. Oncogene 2001, 20:7945-53.

7. Shimada T, Hayes CL, Yamazaki H, et al.: Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 1996, 56:2979-84.

8. Hanna IH, Dawling S, Roodi N, et al.: Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res 2000, 60:3440-4.

9. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:1215.

10. Schaid DJ, Rowland CM, Tines DE, et al.: Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002, 70:425-34.

11. Bethike L, Webb W, Sellick , et al.: Polymorphisms in the cytochrome P450 genes CYP1A2, CYP1B1, CYP3A4, CYP17A1, CYP19A1 and colorectal cancer risk.

12. Landi S, Gemignani F, Moreno V, et al.: A comprehensive analysis of phase I and phase II gene polymorphisms and risk of colorectal cancer. Pharmacogenet Genomics 2005, 15:535-46.

13. Aklillu E, Øvrebø S, Botnen IV, et al.: Characterization of common CYP1B1 variants with different capacity for benzo[a]pyrene-7,8-dihydriodiol epoxide formation from benzo[a]pyrene. Cancer Res 2005, 65:5105-11.

14. Chang BL, Zheng HL, Isaacs SD, et al.: Polymorphisms in the CYP1B1 gene are associated with increased risk of prostate cancer. Br J Cancer 2003, 89:1524-9.

15. Ciciker M, Liu X, Casey G, Witte JS: Role of androgen metabolism genes CYP1B1, P5A/SLK3, and CYP111alpha in prostate cancer risk and aggressiveness. Cancer Epidemiol Biomarkers Prev 2005, 14:2173-7.

16. Tang YM, Green BL, Chen GF, et al.: Human CYP1B1 Leu432Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. Pharmacogenetics 2000, 10:761-6.

17. Shimada T, Gilliam EM, Oda Y, et al.: Metabolism of benzo[a]pyrene to trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase. Chem Res Toxicol 1999, 12:623-9.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/10/420/prepub

doi:10.1186/1471-2407-10-420

Cite this article as: Trubicka et al.: Variant alleles of the CYP1B1 gene are associated with colorectal cancer susceptibility. BMC Cancer 2010 10:420.