EUROPEAN JOURNAL OF CANCER 44 (2008) 854–857

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Could polymorphisms in ATP-binding cassette C3/multidrug resistance associated protein 3 (*ABCC3/MRP3*) modify colorectal cancer risk?

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ARTICLE INFO

Article history:
Received 3 December 2007
Received in revised form
21 January 2008
Accepted 5 February 2008
Available online 7 March 2008

Keywords:
ABCC3
Transporter
Colorectal cancer
Polymorphisms
Susceptibility

ABSTRACT

Multidrug resistance associated protein 3 (ABCC3/MRP3) mediates the efflux of bile salts and several conjugated organic anions out of cells and could be involved in protecting tissues from xenobiotic accumulation and resulting toxicity. In this report, we investigated the hypothesis that a functional missense variant, namely the Arg1297His, and a polymorphism in the promoter region, namely the –211 C > T of the *ABCC3* gene, could be associated with colorectal cancer risk. We did not find any significant association between the two *ABCC3* polymorphisms and colorectal cancer risk.

1. Introduction

Transporters are the gatekeepers for all cells and organelles, controlling uptake and efflux of crucial compounds such as sugars, amino acids, nucleotides, inorganic ions and drugs. It is generally assumed that at least 5% (>2000) of all human genes are transporter-related.[1]

Amongst the most important active transporters, ATP-binding cassette C3/multidrug resistance associated protein 3 (ABCC3/MRP3) is a relatively well-studied member of the ABC transporter family. It has a restricted tissue distribution pattern, being expressed in liver, pancreas, small intestine and colon, with lower amounts of mRNA detected in bladder, kidney, pancreas, lung, spleen, stomach and tonsils.[2–4] In polarised epithelial cells, ABCC3/MRP3 localises to the basolateral membrane. It mediates the efflux of bile salts and several conjugated organic anions out of cells.[5–9] ABCC3/MRP3 role in drug resistance is well-known to be one of the most important amongst ABCs.[10,11] In addition, *ABCC3/MRP3* is expressed in many non-malignant tissues, including the colon, and could be involved in protecting tissues from xenobiotic accumulation and resulting toxicity.[12] *ABCC3/MRP3*-mediated export of conjugates of endogenous substances, such as bilirubin glucoronosides, and xenobiotic substances, such as anti-cancer

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.02.004

drugs, may be involved in the toxicological defence function in various epithelial cell types. Zimmerman and coworkers, in a report exploring the expression of ABCs in the human gut,[13] showed that the *ABCC3* gene is, in large part of the intestine, one of the most expressed transporters of this family. This indicates that mutations in *ABCC3* may critically affect MRP3-mediated pathways of detoxification in human colon, and could thus have a role in cancer susceptibility.

In this report, we investigated the hypothesis that a functional missense variant, namely the Arg1297His, and a polymorphism in the promoter region, namely the –211 C > T of the *ABCC3* gene could be associated with colorectal cancer risk (CRC). We performed a case–control study based on 680 cases and 590 controls from the Czech population. We focused our attention on these two SNPs because of their relative high frequency in the Caucasian population and because amino acid Arg1297 is located close to the second nucleotide binding domain (NBD) and therefore may cause deficient maturation and impaired trafficking of the protein. The polymorphism located in the promoter region is associated with the expression of the gene, as recently reported by Lang.[14] Moreover, this is the most comprehensive study on the *ABCC3* gene polymorphisms which may be relevant pharmacogenetically.

2. Materials and methods

2.1. Study population

A hospital-based case–control study was conducted to study CRC risk. Cases were CRC patients attending nine oncological departments (two in Prague, one each in Benesov, Brno, Liberec, Ples, Pribram, Usti nad Labem and Zlin) distributed in all geographic regions of Czech Republic and covering over half of the population of the country. During the study period (September 2004 to February 2006), a total of 968 cases were diagnosed with CRC in these hospitals. This study includes 680 (70%) patients who could be interviewed and provided biological samples of sufficient quality for genetic analysis. The lost cases were similar to those enrolled with respect to age, sex, tumour location and extent. All cases had histological confirmation of their tumour diagnosis. In the group of cases the genetic testing for hereditary HNPCC was recommended to 4 patients, who belonged to families complying with the Amsterdam criteria II. These patients were excluded from our study.

Controls were selected amongst patients admitted to five large gastroenterological departments (Prague, Brno, Jihlava, Liberec and Pribram) all over the Czech Republic, during the same period of the recruitment of cases. Controls were subjects undergoing colonoscopy for various gastrointestinal complaints. The reasons to proceed to colonoscopy for both cases and controls were (i) macroscopic bleeding; (ii) positive fecal occult blood test (FOBT); (iii) abdominal pain of unknown origin. Due to the high incidence of CRC in the Czech Republic, colonoscopy is largely recommended and practiced, and it is compulsory in case of a positive FOBT. The most common findings for these subjects were haemorrhoids or idiopatic bowel diseases (IBD). Only subjects whose colonoscopic results were negative for malignancy, colorectal adenomas or IBD were chosen as controls. Amongst 739 invited controls, a total of 590 (79.8%) were analysed in this study (lost controls were similar to those included with respect to sex distribution).

Cases included in this study had a median age of 62 years (ranged 27–90), while controls had a median age of 56 years (ranged 28–91). Men were slightly more frequent (57.2% amongst cases and 53.6% of controls).

Study subjects provided information on their lifestyle habits (smoking, drinking, diet, etc.), tentative occupational exposure to xenobiotics and family/personal history of cancer, with the use of structured questionnaires.[15]

Genetic analyses did not interfere with diagnostic or therapeutic procedures for the subjects. All participants signed an informed written consent and the design of the study was approved by the Ethical Committee of the Institute of Experimental Medicine, Prague, Czech Republic.

2.2. DNA extraction and genotyping

DNA was isolated from coded blood samples with standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation, and stored at –80 °C.

Genotyping was carried out using the Taqman assay (Applied Biosystems, Foster City, CA). The MGB Taqman probes were designed using the Primer Express software and synthesised by Applied Biosystems. The reaction mix included 20 ng genomic DNA, 10 pmol for each primer, 2 pmol for each probe and 5 μl of 2× master mix (Applied Biosystems), in a final volume of 10 μl. Thermocycling involved 40 cycles with 30 s at 95 °C followed by 60 s at 60 °C. PCR plates were read on an ABI PRISM 7900HT instrument (Applied Biosystems). Genotype discrimination was performed using SDS software (Applied Biosystems), version 2.2. The PCR profile and reaction conditions were tested and optimised in order to ensure equal contents of template DNA, probes and primers and to allow running with unique thermal conditions. All samples that did not give a reliable result in the first round of genotyping were resubmitted for up to three additional rounds of genotyping. Data points that still remained unfilled after this procedure were left blank.

To ensure quality control, the order of DNA samples from cases and controls was randomised on PCR plates to assure that an equal number of cases and controls could be analysed simultaneously, and all genotyping was conducted by personnel blinded to sample identity. Only genotype calls scored concordantly by two independent trained operators were retained. Finally, 8% of genotypes were repeated for quality control, and yielded a concordance rate of 99.3%. Sequences of primers and probes used for genotyping are available upon request.

2.3. Statistical analysis

The frequency distribution of genotypes was examined for cases and controls. Hardy–Weinberg equilibrium was tested in controls by Fisher's exact test. We used logistic regression for multivariate analyses to assess the main effects of the genetic polymorphism on CRC risk. The primary end-points of the analysis were odds ratios and associated confidence intervals. All the analyses were done with STATA software (StataCorp, College Station, TX).

Table 1 – Associations of ABCC3 functional polymorphisms with colorectal cancer risk

	Cases[a]	Controls[a,b]	OR (95%)[c]	P value	P trend
rs11568591 (Arg1297His)					
A/A	590	548	1		0.74
A/G	67	43	1.44 (0.96–2.11)	0.07	
G/G	1	1	0.92 (0.51–14.44)	0.64	
A/G+G/G	68	44	1.43 (0.97–2.13)	0.07	
rs4793665 (–211 C > T)					
T/T	152	151	1		0.16
C/T	339	280	1.20 (0.91–1.58)	0.18	
C/C	132	104	1.26 (0.89–1.77)	0.18	
C/T + CC	471	384	1.22(0.94–1.58)	0.13	

a Numbers may not add up to 100% of subjects due to genotyping failure. All samples that did not give a reliable result in the first round of genotyping were resubmitted to up to three additional rounds of genotyping. Data points that were still not filled after this procedure were left blank.

b Fisher's exact test for deviation from Hardy–Weinberg equilibrium. *P*-values: 0.58 for rs11568591; 0.22 for rs4793665.

c OR: odds ratio; CI: confidence interval. Adjusted for age and gender.

3. Results

The genotype frequencies amongst the controls and cases were in Hardy–Weinberg equilibrium for both SNPs. The frequencies and distribution of the genotypes and the odds ratios for the associations of the polymorphism are shown in Table 1. We did not find any significant association between *ABCC3* Arg1297His or the –211 C > T polymorphisms and CRC risk, either overall or when subjects were stratified on the basis of gender. If such associations exist, they must be of a smaller magnitude than what would be detectable with our study. Dividing the cases in two groups by an arbitrary cut-off of 50 years at diagnosis, representing late onset versus early onset, we found that individuals who exhibit a late onset and are homozygous carriers for the C allele of the *ABCC3* –211 C > T had an increased risk of CRC (odds ratio 1.48, 95% confidence interval 1.00–2.21, $p = 0.04$). However, stratification by age using the median age at onset (62-years-old) as cut-off did not show any statistically significant difference.

We then divided the patients by the cancer site (colon versus rectum), and we did not find any association with the two polymorphism and the different cancer sites. Moreover, there was no interaction between the age at onset and the cancer site (data not shown).

3.1. *Statistical power*

Our study has 80% power to detect a minimum odds ratio of 2 for the Arg1297His SNP, present with a minor allele frequency (MAF) of 0.04 in our controls, and a minimum odds ratio of 1.4 for the –211 C > T (MAF = 0.46 in the controls of our study), assuming $\alpha = 0.05$, two-sided test and a codominant model.

4. Discussion

Human ABBC3/MRP3 is one of several ABC paralogs localised to the basolateral membrane of polarised cells, and its expression is constitutive in the intestine.[12] Several amino acid variations have been described that modulate the functional properties of ABC transporters. Non-synonymous mutations in NBDs, which are highly conserved domains amongst ABC transporters, may cause deficient maturation and impaired trafficking.[16] The Arg1297His SNP of *ABCC3* has been postulated to exert such an effect.[17] Lang and coworkers[14] showed that the –211 C > T polymorphism of *ABCC3* alters mRNA expression affecting the binding of nuclear factors to the promoter. No other common polymorphisms with established or putative functional relevance are known in this gene.

The previously published functional data made these two polymorphisms attractive candidates for affecting CRC risk. In our case–control study, we did not find any statistically significant association between either polymorphism and CRC risk. Considering the low frequency of the Arg1297His SNP and the available sample size in this study, we cannot exclude that this polymorphism may be associated with a small alteration of risk. Concerning the promoter polymorphism, we observed a non-significant trend in the whole sample set and an increased risk in the individuals with late onset of the disease, suggesting an interaction of the polymorphism and age, although this was not observed when a different age cut-off was used.

In conclusion, our study does not support a major role of Arg1297His and –211 C > T polymorphisms of *ABCC3* gene in risk of CRC. In addition to the data on cancer risk, our study provides information potentially relevant for pharmacogenetics. Most drugs are developed based on data from European-derived 'reference' populations, but clinically relevant DNA polymorphisms often demonstrate population-specific patterns of allele frequencies. The knowledge of the frequency distribution of functional polymorphisms in a population may guide national planning for the selection of therapeutic options. Here, we present, to our knowledge, the largest existing study on two functional common polymorphisms in a key gene such *ABCC3*, which is involved in determining the bioavailability of many drugs.

Conflict of interest statement

None declared.

Acknowledgement

The study was partially supported by Grant GACR 310/05/2626 from the Grant Agency of the Czech Republic.

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