Original article

doi:10.1111/j.1463-1318.2010.02300.x

Ornithine decarboxylase G316A genotype and colorectal cancer risk

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Received 24 September 2009; accepted 15 February 2010; Accepted Article online 29 April 2010

Abstract

Aim Ornithine decarboxylase (ODC) is a modifier of adenomatous polyposis coli-dependent tumourigenesis. The G316 > A polymorphism in intron 1 of the *ODC* gene lies between two *myc*-binding domains and alters the expression of the gene to affect polyamine metabolism. This variant may be associated with recurrence of colorectal adenoma. We examined whether this variant also modified the susceptibility to sporadic colorectal cancer (CRC).

Method The G316 > A variant was analysed in a large (n = 754) CRC case-controlled series of hospital patient volunteers (n = 627) in the Czech Republic, and the relationship with cancer risk was estimated by conditional logistic regression.

Results After adjusting for age and sex, G316 > A was associated with no decrease in CRC risk for either heterozygotes [odds ratio 0.98, 95% confidence interval (CI) 0.77–1.23] or rare allele homozygotes (odds ratio 0.92, 95% CI 0.61–1.37).

Conclusion The G316 > A functional variant in the *ODC* gene is unlikely to make much impact on reducing CRC risk regardless of the reduction in risk found for the recurrence of colorectal adenoma.

Keywords Colorectal cancer, cancer genetics, association study, ornithine decarboxylase cancer prevention, colorectal adenoma, aspirin chemoprevention

Introduction

Ornithine decarboxylase (ODC) is the primary rate-limiting enzyme in the polyamine synthesis pathway by urea cycle decarboxylation of the amino acids ornithine and arginine [1]. Polyamines affect several key carcinogenic processes, and their inhibition is associated with reduced cell proliferation, increased apoptosis, and suppression of angiogenesis and cancer development in animal models [2–5]. Expression of *ODC* and levels of polyamines are increased in colon cancer and in many other human epithelial tumours [2,4–6]. Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) exert chemopreventive effects on colorectal neoplasia, perhaps partly via their influence on polyamine levels by induction of polyamine catabolism [2,3]. A common early event in colorectal tumourigenesis is loss of adenomatous polyposis coli (APC) function and the overexpression of *MYC* oncogene, resulting in increased synthesis of ODC, a target for the transcription factor MYC [7]. Altered expression of *ODC* because of its G316A polymorphism (rs2302615) has been shown to affect polyamine metabolism. Two promoter region transcription factor-binding sites bracket the functional *ODC* G316A variant, with the minor allele reducing ODC enzyme expression [8,9]. Thus, the *ODC* G316A genotype may influence the risk of development of colorectal neoplasia.

Hubner *et al.* [10] typed the G316A variant in 546 individuals from a UK prevention trial of aspirin for colorectal adenoma (CRA) recurrence. They found that the rare 316AA homozygotes had a nonsignificantly reduced CRA recurrence risk [relative risk (RR) 0.43, 95% confidence interval (CI) 0.16–1.15], with a suggestion of a possibly lower recurrence risk if exposed also to aspirin (RR 0.24, 95% CI 0.03–1.71). To help clarify whether these preliminary results indicated a real effect, they further pooled their findings with data from the two

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previous USA studies [8,11] so that in 2207 individuals, those with *ODC* 316AA genotype were at a significantly reduced CRA recurrence risk regardless of aspirin use (RR 0.68, 95% CI 0.47–0.99; $P = 0.04$). Following stratification by genotype and aspirin exposure, individuals with homozygous wild-type or heterozygous genotypes derived modest benefit from aspirin (RR 0.85, 95% CI 0.72–1.01), whereas in those with both *ODC* 316AA genotype and aspirin exposure, recurrence risk was halved (RR 0.52, 95% CI 0.29–0.91; $P = 0.02$).

To our knowledge, there are no reports of this variant having been examined as a potential genetic modifier of colorectal cancer (CRC) susceptibility. We wished to know whether this variant was also associated with CRC development and tested this in a large case ($n = 754$)–control ($n = 627$) series from the Czech Republic, a country that currently has the highest CRC mortality rate in the developed world [12].

Method

Study population

The study population comprised 754 incident cases with CRC and 627 hospital-based healthy control subjects. The cases included 509 colon cancers, 210 rectal cancers and 35 individuals for whom the cancer site was not specified. Eligibility criteria for study participation of cases and control subjects included Czech origin, age 29 years or more and consent to provide biological samples for genetic analysis. To reduce selection bias, only those subjects with no previous diagnosis were included in the study to avoid inclusion of patients with chronic diseases who may be repeatedly admitted to hospital and modify their habits because of their disease.

The study cases with positive colonoscopy results for malignancy, confirmed by histology as colon or rectal carcinomas, were recruited between September 2004 and February 2006 from patients visiting nine oncological departments throughout the Czech Republic (two in Prague; the others in Benesov, Brno, Liberec, Ples, Pribram, Usti, Labem and Zlin). During the study period, sixteen individuals were excluded because they met the Amsterdam criteria I and II for hereditary CRC [13,14]. Control subjects were selected during the same period from individuals undergoing colonoscopy for various gastrointestinal complaints (macroscopic bleeding, positive faecal occult blood test or abdominal pain of unknown origin) from five large gastroenterological departments (Prague, Brno, Jihlava, Liberec and Pribram). Control subjects were selected from those showing a negative colonoscopy for malignancy or functional bowel disease. The study group were accrued for previous case-controlled studies investigating the risk of genetic polymorphisms as recently described [15]. One hundred and twelve control subjects were excluded from initial groups because the eligibility criteria were not met or information on lifestyle and potential risk factors, or DNA, was not available. In the end, of 739 recruited control subjects, a total of 627 (84.8%) were included in the study. The participating subjects gave written informed consent in accordance with the Declaration of Helsinki. The design of the study was approved by the Ethical Committee of the Institute of Experimental Medicine, Prague.

Study questionnaire

All subjects were interviewed by trained personnel using a structured questionnaire to determine demographic characteristics and potential risk factors for CRC. Study subjects provided information on their living area, education, lifestyle habits (smoking, drinking, diet, etc.), body mass index (BMI), diabetes, family/personal history of cancer and long term (at least 6 consecutive months) drug use. The median age of the cases was 61 years (range 27–85) and of the control subjects 53 years (range 29–91). The characteristics of the study population have been reported previously [15,16].

Genotyping

Germline DNA was isolated from peripheral leucocytes within 4 weeks after the blood sample was collected using the standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation and stored at −80°C. *ODC* G316A genotyping was performed simultaneously for cases and control subjects by *KBioscience* (Hoddesdon, Hertfordshire, UK) using a competitive allele-specific PCR system (KASPar). The genotyping success rate was 97.25%, as there were 38 failed calls (18 for cases and 20 for control subjects) out of the 1381 total attempted reactions. The genotyping assay was validated using a random 10% of samples as duplicate quality controls with complete concordance. Samples with unclear or failed genotype calls were excluded from the analysis. Details of allele probe sequences are available on request.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was tested for the *ODC* G316A polymorphism in the control subjects using the χ^2 test. Age- and sex-adjusted unconditional logistic regression was used to estimate the cancer risk

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association with 95% confidence intervals for the *ODC* G316A single nucleotide polymorphism (SNP) (SAS statistical software, version 9; SAS Institute, Cary, North Carolina, USA). For all analyses, the reference genotype was defined as the homozygous (wild-type) allele. All main effects analysis models were run for colon and rectum combined (i.e. CRC) as well as separately. Tests for linear trend were performed using a score variable with values from 1 to 3 consistent with the genotype groupings. Using the Quanto software [17], we calculated that with our present sample size, our study had 80% power ($\alpha = 0.05$) to detect odds ratio risk reductions of 0.7 and 0.5 in the dominant and recessive models, respectively, for the *ODC* G316A SNP with a minor allele frequency of 30%.

Results

The frequency for the rare *ODC* 316A allele is comparable in this Czech control group (0.30) to that in the UK (0.24) [10] and the US cohorts (between 0.25 and 0.27) [8,11] previously studied for the relationship with CRA recurrence.

There was no association between the G316A variant with the risk of CRC in 737 cases and 607 control subjects (Table 1). Thus, there appeared to be no risk of cancer with this polymorphism alone as previously published for adenoma recurrence [10]. The meta-analysis proposed an association of *ODC* 316AA with decreased risk of recurrence of CRA in 2207 individuals from the USA and UK (RR 0.68, 95% CI 0.47–0.99). We estimated that heterozygotes of *ODC* 316GA or 316AA rare allele homozygotes alone had no significant differences in their risk for CRC in the Czech population (OR 0.98, 95% CI 0.77–1.23 and OR 0.92, 95% CI 0.61–1.37, respectively; *P* for trend = 0.68). Furthermore, no significant associations were found for colon or rectal cancers separately, or for men and women or for other variables, such as BMI, smoking, alcohol consumption or the presence of polyps.

Table 1 *ODC* intron 1 G316 > A genotype and risk of colorectal cancer.

ODC (G316 > A) rs2302615 genotype	Cases (numbers)	Controls (numbers)	OR (95% CI)*	*P*-value
GG	377	303	1.00 (reference)	–
GA	297	247	0.98 (0.77–1.23)	0.85
AA	62	57	0.92 (0.61–1.37)	0.66

*Age and sex adjusted.

Discussion

The aim of this study was to determine whether the functional *ODC* G316A polymorphism influences susceptibility to sporadic CRC. We studied the association of this variant with the risk of CRC using a hospital-based case-controlled design with 754 patients and 627 control subjects. There was no significant association between *ODC* G316A and risk of CRC for either heterozygotes (OR 0.98, 95% CI 0.77–1.23) or rare allele homozygotes (OR 0.92; 95% CI 0.61–1.37). Furthermore, there were no significant differences between site of cancer (colon or rectum), sex, BMI, smoking, alcohol or the presence of polyps.

Studies linking polyamine metabolism and the development of CRC precursor lesions have suggested that this pathway may play an important role in CRC carcinogenesis [2]. The relationship between *ODC* G316A genotype, aspirin use and CRA recurrence has been investigated in three previous studies. Martinez *et al.* [8], in a randomized trial of wheat bran fibre intervention for CRA recurrence in the USA, reported that aspirin users homozygous for the minor A-allele of the G316A variant of the *ODC* gene were approximately 0.10 times as likely to have an adenoma recurrence as non aspirin users homozygous for the major G-allele. However, only 209 (30%) of the 688 individuals genotyped for *ODC* G316A reported aspirin use and of those, only 11 harboured the AA genotype. Thus, the confidence intervals were wide (RR 0.10, 95% CI 0.02–0.66). In a more pertinent study by Barry *et al.* [11], the *ODC* G316A genotype was analysed from 973 participants in the Aspirin/Polyp Prevention Study who were randomly assigned to placebo or aspirin treatment (81 or 325 mg daily) and followed for 3 years for the occurrence of new adenomas. Although no association was found between *ODC* genotype and the occurrence of new adenomas overall, aspirin treatment reduced the adenoma risk in subjects with at least one A-allele (RR 0.77, 95% CI 0.63–0.95; $P = 0.02$). In a pooled analysis of these data with their UK cohort for a total of 2207 individuals, Hubner *et al.* [10] reported a significantly decreased risk of CRA recurrence for *ODC* 316AA (RR 0.68, 95% CI 0.47–0.99) and an even further decreased risk of approximately half for *ODC* 316AA and aspirin intake (RR 0.52, 95% CI 0.29–0.91). The authors concluded that the *ODC* G316A genotype was prognostic for CRA recurrence and had the potential to be a clinically useful genetic marker to identify individuals likely to derive the greatest benefit from aspirin chemoprevention.

Our sample numbers are of the same order as the three separate adenoma studies combined by Hubner *et al.* [10], where a nonsignificant risk reduction was found in

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each before the larger, pooled analysis. If this *ODC* variant does have a protective effect on CRC risk, the effect is likely to be more modest than that reported by Hubner *et al.* [10], which could be understood in the context that not all adenomas are at high risk of progressing to cancers. Our study was not sufficiently powered to test for a smaller decreased risk for cancers than the adenomas previously reported [10] (RR 0.68 for 316AA), as there was an 80% power to detect a reduction in the odds ratio risk of 0.7 and 0.5 in the dominant and recessive models, respectively, for the *ODC* G316A SNP with a minor allele frequency of 30%. It is possible, therefore, that the *ODC* 316AA rare allele homozygotes do have a more modest reduced CRC risk. Together with the biological evidence of the functional effect of this variant and the similarity in results from the UK adenoma risk study to our CRC risk investigation, this would suggest there may yet be a role of the *ODC* G316A variant in the adenoma–cancer pathway. Recently, Zell *et al.* [18], in a population-based study of 400 cases, reported that the CRC-specific survival hazards ratio was 2.02 (95% CI 1.17–3.50, $P = 0.012$) for *ODC* GA/AA genotypes. Therefore, although the *ODC* G316A variant may be protective for colon adenoma recurrence (CRA) and detrimental for survival of colon cancer (CRC), it is unlikely to play a major role in susceptibility to CRC development.

We have no data on aspirin use in the Czech case–control series. There is mounting evidence for a chemopreventive effect of aspirin and nonsteroidal anti-inflammatory drugs against the development and recurrence of colorectal neoplasia [19–21,2]. However, as some individuals experience side-effects, the use of these agents in the prevention of CRC is not currently recommended [22,23]. As *ODC* genotype modifies the response to aspirin treatment for CRA prevention [8,10,11], the potential of positively modifying aspirin CRC chemoprevention and selecting individuals genetically favourable to benefit from this treatment suggests that it may be worthwhile to test this variant in a much larger sample size of CRAs and CRCs.

Acknowledgements

This study was supported by the following grants: Czech Science Foundation, no. GACR 310/07/1430 and no. GACR 305/09/P194; and grant of the Grant Agency of Charles University in Prague, no. GAUK 15109/2009.

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