

CYP7A1 promoter polymorphism –203A>C affects bile salt synthesis rate in patients after ileal resection

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Abstract Cholesterol 7 α -hydroxylase (CYP7A1) plays a crucial role in cholesterol metabolism and has been implicated in genetic susceptibility to atherosclerosis. Thus, an understanding of its transcriptional regulation is of considerable importance. We evaluated the effect of a common –203A>C polymorphism in the CYP7A1 promoter region on the activity of CYP7A1, estimated as the ratios of serum 7 α -hydroxycholest-4-en-3-one (C4) to either total or non-HDL-cholesterol. The study was performed on patients after resection of the distal ileum, leading to upregulation of CYP7A1 activity (n = 65). Healthy volunteers served as the control group (n = 66). Whereas higher CYP7A1 activity was associated with the –203A allele in the patient group (C4/cholesterol ratio, 29.0 vs. 14.8 μ g/mmol, P = 0.032; C4/non-HDL-cholesterol ratio, 53.3 vs. 21.3 μ g/mmol in –203AA and –203CC, P = 0.017, respectively), no differences were observed in the healthy controls. We conclude that under physiological conditions, the –203A>C polymorphism in the CYP7A1 gene promoter region does not seem to have any clinically relevant effect. However, in patients with severe bile salt malabsorption, this polymorphism markedly affects CYP7A1 activity.—Leníček, M., V. Komárek, M. Zimolová, J. Kovář, M. Jirsa, M. Lukáš, and L. Vitek. CYP7A1 promoter polymorphism –203A>C affects bile salt synthesis rate in patients after ileal resection. *J. Lipid Res.* 2008. 49: 2664–2667.

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Conversion of cholesterol into bile salts (BSs) represents an important cholesterol-biotransforming pathway. BS biosynthesis is initiated by cholesterol 7 α -hydroxylase (CYP7A1, EC 1.14.13.17), the rate-limiting enzyme in the classical BS biosynthetic pathway. The considerable contribution of CYP7A1 in the regulation of cholesterol metabo-

lism has been confirmed in both animals and humans, where higher CYP7A1 activity correlated with lower cholesterol levels, and vice versa (1–6). Therefore, genetic determinants of its activity are of special interest. One of the most studied candidates is the –203A>C polymorphism (c.–267A>C, dbSNP rs3808607) in the promoter region of CYP7A1. This common variant represents the haplotype block, which covers a substantial part of the promoter and the first exon of CYP7A1 (7).

Wang et al. (8) described an association between the –203A>C polymorphism and plasma LDL-cholesterol levels. Since then, numerous studies have been performed to examine the importance of this polymorphism or a linked variant, –469C>T (c.–533C>T, dbSNP rs3824260) in cholesterol metabolism regulation (9–14). The results were, however, inconsistent. Carriage of the –203C allele has been reported to be associated with increased LDL-cholesterol in men, but not in women (9), with increased serum levels of apolipoprotein A-I but not with apolipoprotein B-100 (10), and with significant LDL-cholesterol changes after dietary intervention (11, 12). In a study by Hegele et al. (13) the –203A allele was associated with higher LDL-cholesterol in Keewatin Inuits and lower HDL-cholesterol in Hutterites, whereas no association has been found in Sandy Lake Oji-Creek members. All of these studies correlated the presence of –203A>C variants with plasma cholesterol levels, rather than with CYP7A1 activity. Only Abrahamsson et al. (14) measured CYP7A1 activity or plasma levels of 7 α -hydroxycholest-4-en-3-one (C4), a marker of CYP7A1 activity (15) in two small cohorts (of 21 and 30 subjects), respectively. Based on these observations, as well as on in vitro experiments, Abrahamsson et al. (14)

Abbreviations: BS, bile salt; CYP7A1, cholesterol 7 α -hydroxylase; C4, 7 α -hydroxycholest-4-en-3-one.

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concluded that these polymorphisms contributed neither to CYP7A1 activity nor to the plasma LDL-cholesterol concentration in humans.

The aim of the present study was to ascertain, whether the $-203A>C$ polymorphism in the *CYP7A1* promoter affects the activity of CYP7A1 in patients with a resection of the distal ileum, in whom the BS synthesis is grossly upregulated.

MATERIALS AND METHODS

Subjects

Two groups of subjects were included in the study. The first group consisted of healthy volunteers, employees of 1st Faculty of Medicine, Charles University in Prague, and blood donors. The other group consisted of inflammatory bowel disease patients with resection of the distal ileum. Owing to the lack of ileal bile acid transporter (ASBT, SLC10A2), BS malabsorption with a compensatory increase of CYP7A1 activity was expected in this group (6). Additionally, fibroblast growth factor 19, a suppressor of CYP7A1, is produced in the small intestine under physiological conditions (16–18). After ileal resection, its production is expected to decrease, with subsequent derepression of CYP7A1. The length of the resected ileum was classified as extensive (≥ 70 cm) or small (< 70 cm). Patients were recruited at the 4th Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague and at IBD Clinical and Research Center, ISCARE I.V.F. in Prague. Individuals taking BSs or BS sequestrants, which might influence the activity of CYP7A1, were excluded. Basic demographic characteristics of the subjects are shown in **Table 1**.

All subjects were genotyped for the $-203A>C$ variant. The sera of homozygotes were further analyzed. The study was approved by the local Ethics Committee, and all participants gave their written informed consent prior to enrollment.

C4 and cholesterol measurements

Serum samples were obtained in the morning, aliquotted, and stored at -80°C until analysis. C4 concentration was measured by HPLC, as previously described (19). Briefly, 1 ml of serum and 30 ng of internal standard (7 β -hydroxycholest-4-en-3-one; Steraloids,

Newport, RI) were extracted by chloroform-methanol, purified on a Strata SI 100 mg precolumn (Phenomenex, Torrance, CA), and separated by HPLC (HP 1100 series, Agilent Technologies, Santa Clara, CA). The column (SGX C18, 4×250 mm, particles, $4 \mu\text{M}$) was manufactured by Tessek, Prague, Czech Republic; mobile phase, acetonitrile-water (95:5, v/v), 1 ml/min, 20°C ; detection/reference wavelength, 241/360 nm.

Total and HDL-cholesterol levels were measured on a modular automatic analyzer (Roche Diagnostics, Basel, Switzerland) using commercially available kits (CHOLESTEROL liquicolor and HDL CHOLESTEROL liquicolor, respectively, both by Human GmbH, Wiesbaden, Germany).

The activity of CYP7A1 was estimated using a serum C4/total cholesterol concentration ratio, which represents a more accurate marker of CYP7A1 activity than does serum C4 level alone (20). Additionally, the C4/non-HDL-cholesterol ratio was also used.

DNA analysis

Genomic DNA was isolated from peripheral blood white cells by a standard salting-out method (21). The $-203A>C$ locus was genotyped by PCR-*BsaI* restriction fragment length polymorphism; forward primer 5'-ATTAGCTATGCCCATCTTAAACAGG-3' and reverse primer 5'-TAACTGGCCTTGAAGTAAGTCCAC-3' were used for PCR amplification of the corresponding DNA fragment.

Statistical analyses

Normally distributed data were compared using the Student *t*-test and presented as the mean (\pm SD), whereas skewed data were compared using the Mann-Whitney Rank Sum test and presented as the median (5–95%). The Hardy-Weinberg equilibrium was tested by the χ^2 test. Analyses were performed using STATISTICA software (version 8). The *P* value < 0.05 was considered as significant.

RESULTS

As expected, C4/cholesterol as well as C4/non-HDL-cholesterol ratios in patients after ileal resection were higher than in controls (20.4 vs. 2.3 and 33.2 vs. 3.7 $\mu\text{g}/\text{mmol}$, respectively, $P < 0.001$ for both analyses). Both total and

TABLE 1. Effect of the $-203A>C$ polymorphism on CYP7A1 activity

	$-203AA$	$-203CC$	<i>P</i>
Controls (n = 66)			
Gender (f/m)	7/16	28/15	0.020
Age (years)	41.0 ± 13.4	43.7 ± 11.8	0.470
C4/cholesterol	2.6 (1.0–5.5)	2.3 (0.6–7.5)	0.462
C4/non-HDL-cholesterol	4.2 (1.4–10.1)	3.6 (0.9–12.7)	0.548
All patients (n = 65)			
Gender (f/m)	21/16	15/13	1.000
Age (years)	41.3 ± 12.2	42.7 ± 12.6	0.810
C4/cholesterol	29.0 (3.5–106.4)	14.8 (2.4–68.9)	0.032
C4/non-HDL-cholesterol	53.3 (3.4–166.7)	21.3 (2.7–125.9)	0.017
Patients with resection < 70 cm (n = 57)			
C4/cholesterol	24.3 (2.2–77.1)	13.5 (1.9–75.5)	0.112
C4/non-HDL-cholesterol	43.5 (3.3–128.2)	18.9 (2.7–125.9)	0.059
Patients with resection ≥ 70 cm (n = 8)			
C4/cholesterol	108.9 (81.0–132.2)	26.0 (4.4–31.9)	0.012
C4/non-HDL-cholesterol	157.3 (118.1–186.1)	43.9 (9.1–48.6)	0.029

Results are given as mean \pm SD, or as median (5–95%) when data were not normally distributed. Distribution of genotypes in both controls and patients followed Hardy-Weinberg equilibrium. The 7 α -hydroxycholest-4-en-3-one (C4)/cholesterol and C4/non-HDL-cholesterol ratios are expressed in $\mu\text{g}/\text{mmol}$. Boldface indicates statistically significant results.

non-HDL serum cholesterol levels were significantly higher in controls than in patients (5.03 vs. 4.12 mmol/l and 3.25 vs. 2.69 mmol/l, respectively, $P < 0.001$ for both analyses).

In the group of all patients, the $-203AA$ genotype was associated with a higher C4/cholesterol ratio, compared with the $-203CC$ genotype (Table 1). Even more pronounced differences were observed in the subgroup of patients after extensive resection of the ileum; C4/cholesterol ratio in $-203AA$ homozygotes was significantly higher than in $-203CC$ patients (Table 1). The difference between the C4/cholesterol ratio in $-203AA$ and CC homozygotes after short ileal resection showed the same tendency; however, it did not reach statistical significance (Table 1).

Similarly, C4/non-HDL-cholesterol ratios were significantly higher in $-203AA$ than in $-203CC$ homozygotes in all patients (Fig. 1), as well as in patients after extensive ileal resection (Table 1). In patients after short resection, the difference between $-203AA$ and $-203CC$ homozygotes did not reach statistical significance.

No significant effect of the $-203A>C$ polymorphism in either C4/cholesterol or C4/non-HDL-cholesterol was found in the control group (Table 1).

DISCUSSION

Because of the key role of CYP7A1 in cholesterol metabolism, regulation of its activity is an important issue. Despite intensive research, the role of the $-203A>C$ variant in the modulation of CYP7A1 activity has not yet been explained. To our knowledge, there is no direct evidence supporting the association of this variant with CYP7A1 activity in humans. Abrahamsson et al. (14) did not find any association between the $-203A>C$ genotype and hepatic CYP7A1 activity in gallstone disease patients. The number of subjects in this study was, however, limited, and no $-203AA$ homozygotes were included. Similarly, the authors did not ob-

serve any association between the $-203A>C$ variant and serum levels of C4 as a marker of CYP7A activity in a cohort of 30 subjects with asymptomatic gallstone disease.

Because direct measurement of CYP7A1 activity requires liver biopsy, less-invasive markers are preferred in humans. It has been shown that serum levels of C4 reflect CYP7A1 activity (15, 22). Honda et al. (20) suggested that the C4/cholesterol ratio would be a more accurate serum marker, because C4 is transported in lipoprotein particles carrying cholesterol. The authors experimentally confirmed that the C4/cholesterol ratio, as a marker of CYP7A1 activity, is superior to serum C4 levels. Based upon the fact that cholesterol for BS synthesis recruits predominantly from non-HDL-cholesterol (23, 24) and probably enters the circulation in the VLDLs (the first members of the metabolic lipoprotein cascade that includes all the lipoproteins other than HDL), the C4/non-HDL-cholesterol ratio (product/substrate) might theoretically be even more accurate.

In our study, we report an association of CYP7A1 activity with the $-203A>C$ variant in patients after resection of the distal ileum. Owing to BS malabsorption and the possible lack of fibroblast growth factor 19, originating predominantly from the ileum, these patients have upregulated CYP7A1 activity (6, 16–18). Our patients, homozygous for the $-203A$ allele, had an approximately 2-fold higher C4/cholesterol or C4/non-HDL-cholesterol ratio than did the $-203CC$ homozygotes. The difference was more pronounced in patients after extensive resection. In patients after a small (<70 cm) resection of the ileum, both ratios were 2-fold higher in homozygotes for $-203A$, when compared with $-203CC$ carriers. The differences, however, did not reach statistical significance. This is probably owing to the large heterogeneity of this group, in which patients after considerable resection as well as those with clinically negligible resection are included. In accord with Abrahamsson et al. (14), we did not observe significant differences in the C4/cholesterol or C4/non-HDL-cholesterol ratios between $-203AA$ and $-203CC$ homozygotes in the control group.

We hypothesize that under physiological conditions, the functional reserve of CYP7A1 is sufficient to override the effect of the promoter region, and that its variants do not contribute substantially to the CYP7A1 activity. However, when CYP7A1 is upregulated (e.g., in patients with ileal resection leading to malabsorption of BS), the $-203C$ promoter is not able to increase the transcription level as much as the $-203A$ is.

The higher activity of CYP7A1 leads to consumption of cholesterol within hepatocytes, which subsequently increases expression of LDL receptors and thus lowers the serum LDL-cholesterol levels (6, 25, 26). Similarly, lower CYP7A1 activity should result in higher serum cholesterol levels. In the present study, we did not focus on the serum cholesterol levels, because in the healthy population, the effect of the $-203A>C$ polymorphism on cholesterol levels seems to be minor if any (8–14), and a large cohort needs to be collected in order to draw a relevant conclusion. Furthermore, in patients after ileal resection, the different nutritional status represents an important confounding

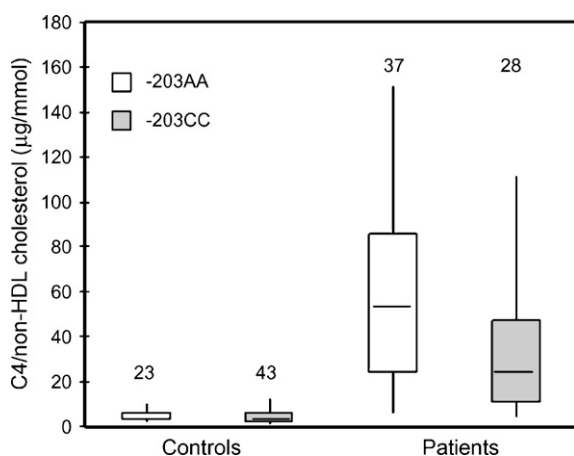


Fig. 1. 7α -Hydroxycholest-4-en-3-one (C4)/non-HDL-cholesterol ratio in $-203AA$ and $-203CC$ homozygotes. CYP7A1 activity estimated as C4/non-HDL-cholesterol ratio is higher in patients homozygous for the $-203A$ allele. Data are expressed as median, 25–75% (boxes) and 5–95% (whiskers). Number of subjects is indicated.

factor, which would mask the possible effect of the polymorphism on serum cholesterol levels.

In conclusion, our results suggest, that the −203AA genotype is associated with higher activity of CYP7A1 compared with −203CC in subjects with upregulated activity of CYP7A1. Under physiological conditions, however, the effect seems to be negligible.

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