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Plasma carnitine ester profiles in Crohn's disease patients characterized for SLC22A4 C1672T and SLC22A5 G-207C genotypes

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Crohn's disease (CD) is a chronic inflammatory bowel disorder caused by environmental and genetic factors. The purpose of this study was to analyse the possible influence of functional variants of genes of OCTN cation transporters on the carnitine ester profile of patients with CD. Genotyping for SLC22A4 1672C → T, SLC22A5-207G → C mutations and three common NOD2 variants (R702W, G908R and 1007finsC) were performed in 100 adult CD patients and in ninety-four healthy controls by direct sequencing. The carnitine ester profile was determined using ESI triple quadrupole tandem MS. Contrary to the NOD2/CARD15 mutations, none of the SLC variants showed increased prevalence in the CD group, the prevalence of TC haplotype did not differ between the patients and the controls. In the mixed group of CD patients the fasting propionyl- (0·243 (SEM 0·008) *v.* 0·283 (SEM 0·014) μmol/l), butyryl- (0·274 (SEM 0·009) *v.* 0·301 (SEM 0·013)) and isovalerylcarnitine (0·147 (SEM 0·006) *v.* 0·185 (SEM 0·009)) levels were decreased; while the level of octenoyl- (0·086 (SEM 0·006) *v.* 0·069 (SEM 0·005)), myristoleyl- (0·048 (SEM 0·003) *v.* 0·037 (SEM 0·003)), palmitoyl- (0·140 (SEM 0·005) *v.* 0·122 (SEM 0·004)) and oleylcarnitine (0·172 (SEM 0·006) *v.* 0·156 (SEM 0·008); $P<0·05$ in all comparisons) were increased. After sorting the patients into SLC22A genotype-specific subgroups, no significant differences could be observed between them. The carnitine ester profile data suggest selective involvement of the carnitine esters in CD patients, probably due to their altered metabolism.

Carnitine ester profile: OCTN1: OCTN2: NOD2/CARD15: Crohn's disease

Crohn's disease (CD) is a chronic relapsing inflammatory disease affecting the lower end of the ileum and often involving the colon and other parts of the gastrointestinal tract. The development of this disease is known to be influenced by both environmental factors and genetic predisposition. The most important susceptibility gene for CD was the CARD15 (NOD2) gene on chromosome 16 (Hampe *et al.* 2001; Hugot *et al.* 2001; Ogura *et al.* 2001). Three disease-associated variants, the R702W, G908R and the 1007finsC, have been found frequently in different populations.

L-Carnitine plays an essential role in mitochondrial β-oxidation of fatty acids, since it is a cofactor for shuttling long-chain fatty acids across the inner mitochondrial membrane (Fritz & Yue, 1963; Bremer, 1983). Moreover, it is also capable of forming esters with several medium-chain fatty acids and SCFA of both endogenous and exogenous origins (Melegh *et al.* 1987; Bieber, 1988). In mammals, carnitine homeostasis is maintained by endogenous synthesis, absorption from dietary sources and efficient tubular reabsorption by the kidney.

Recently Peltekova *et al.* (2004) found two polymorphisms in the carnitine/organic cation transporter gene cluster (SLC22A4 and SLC22A5, encoding OCTN1 and OCTN2, respectively), that gave rise to susceptibility for CD. As the functional variants in general can have an effect on the carnitine homeostasis, the goal of the present study was to investigate the possible influence of the SLC22A genotypic variants on the circulating carnitine ester profile in CD patients.

Materials and methods

Patients

We examined 100 patients with CD (forty-seven males, fifty-three females, mean age 37·3 years, range 18–87 years), and ninety-four carefully selected clinically healthy age-, sex-, weight- and height-matched control subjects (forty-seven males, forty-seven females, mean age 45·6 years, range 23–80 years).

Abbreviations: CD, Crohn's disease; ESI, electrospray ionization.

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Diagnosis of the disease relied upon the history of the patients, clinical symptoms, negative stool examination for bacteria and parasites; and histology results of colonoscopy. Exclusion criteria in both groups were as follows: secondary causes of small intestine and/or colonic disease, systemic diseases, any malformations, evidence of intestinal bacterial or other infection, history or evidence of any inherited metabolic disease, hepatic or renal disease, and pregnancy.

The clinical and laboratory data are results of measurements performed from sample aliquots of blood collected after an overnight fast precisely between 08.00 and 08.30 hours, both in the CD patients and in the healthy control subjects. This strict post-alimental time scheduling was introduced to prevent the diet- or fasting time-induced dynamic changes of carnitine esters in the circulation (Costa *et al.* 1999).

Informed consent was obtained from each participant of the study and the study design was approved by the local Ethics Committee.

ESI–MS–MS analysis

In the majority of the CD patients (*n* 85) and in a group of the controls (*n* 45) we determined the acylcarnitines as butyl esters by an isotope dilution MS method using a Micromass Quattro Ultima ESI triple–quadrupole mass spectrometer (Manchester, UK). The procedure was principally the method described previously (Bene *et al.* 2005). Briefly, 10 μl plasma was spotted and dried on to a filter paper and free carnitine and acylcarnitines were extracted with 200 μl methanol containing internal deuterium-labelled standards (0·76 μmol/l [$^2H_3$]free carnitine, 0·04 μmol/l [$^2H_3$] propionylcarnitine, 0·04 μmol/l [$^2H_3$]octanoylcarnitine and 0·08 μmol/l [$^2H_3$]palmitoylcarnitine). After 20 min of agitation the supernatant was evaporated to dryness under nitrogen at 40°C, and subsequently derivatized in 100 μl 3 mol/l butanolic HCl for 15 min at 65°C. Samples were evaporated to dryness again under nitrogen at 40°C and redissolved in 100 μl of the mobile phase (acetonitrile–water, 80 : 20). A 10 μl aliquot of each sample was introduced at a flow rate of 100 μl/min into the ESI cone using the Waters (Milford, MA, USA) 2795 HPLC system. Free carnitine and all acylcarnitines were measured by positive precursor ion scan of *m/z* 85 (scan range 200–550 *m/z*) using the following settings: capillary voltage 2·50 kV, cone voltage 55 V and collision energy 26 eV. Total analysis time was 4 min per sample and each sample was measured in triplicate starting with the injection step, and the results are the means of the three determinations.

Our mass spectrometry facility is in the International Newborn Screening Quality Assurance Program organized by the Center for Disease Control and Prevention, USA (http://www2.cdc.gov/nceh/NewbornScreening/). This activity involves controlled measurements of selected acylcarnitines in two quality control regimens and four proficiency tests per year.

Statistics

For statistics the Student's *t*-test for unpaired samples was used. The values are expressed as means and their standard errors, in three decimals for the carnitine esters with respect to the low levels in the case of the long-chain carnitine esters.

DNA sequencing

To detect the OCTN and NOD2 variants we carried out direct sequencing by means of a BigDye Terminator labelling system with ABI 3100 automatic sequencer (Foster City, CA, USA) with a slightly modified method described previously. Genomic DNA from index patients and controls was isolated from peripheral blood using a standard desalting procedure. For the PCR amplification as well as for the sequencing the following primers were used: for R702W the forward primer was 5′-GAGCCGCACAACCTTCAGATC-3′, and the reverse primer was 5′-ACTTGAGGTGCCCAACAT-TCAG-3′; for G908R the forward primer was 5′-GTTCATG-TCTAGAACACATATCAGG-3′, and the reverse primer was 5′-GTTCAAAGACCTTCAGAACTGG-3′; for 1007finsC the forward primer was 5′-CCTTGAAGCTCACCATTGT-ATC-3′, and the reverse primer was 5′-GATCCTCAAAAT-TCTGCCATTC-3′; for SLC22A4 C1672T the forward primer was 5′-AGAGAGTCCTCCTATCTGATTG-3′, and the reverse primer was 5′-TCCTAGCTATTCTTCCATGC-3′; for SLC22A5 G-207C the forward primer was 5′-AGTC-CCGCTGCCTTCCTAAG-3′, and the reverse primer was 5′-GTCACCTCGTCGTAGTCCCG-3′. The PCR was carried out in a final volume of 50 μl containing 200 μM of each dNTP, 2 units of Taq polymerase, 5 μl reaction buffer (100 mM-Tris-HCl, pH 9·0; containing 500 mM-KCl, 15 mM-$MgCl_2$), 0·2 μM of each primer and 1 μg DNA to be amplified. The amplification was performed for a total of thirty-five cycles with the use of an MJ Research (Waltham, MA, USA) PTC-200 thermal cycler. The conditions were: predenaturation for 2 min at 95°C, denaturation 30 s at 95°C, annealing 30 s at 50°C for CARD15 mutations, 54°C for SLC22A4 and 58°C for SLC22A5 variants, primer extension 30 s at 72°C, the final extension at 72°C lasted for 5 min.

For statistics the χ^2 method (cross-table analyses) was used to analyse the possible associations with mutations.

Results

The allele frequencies are shown in Table 1. A total of 32 % of CD patients carried at least one NOD2 mutation compared to 12·8 % of controls ($P<0·005$). With respect to the 1007finsC mutation, 17 % of CD patients were either heterozygous or homozygous compared to 4·3 % of controls ($P<0·005$). Generally, the R702W and G908R were also observed in higher frequencies in patients compared to controls. There was no statistically significant difference in the prevalence of TC haplotype between the CD patients and controls (Table 1).

The plasma circulating carnitine ester profiles in the mixed CD population are shown in Table 2. The plasma level of free carnitine and acetyl carnitine did not differ between the CD patients and the controls. By contrast, significant decreases were found in fasting propionyl-, butyryl- and isovalerylcarnitine ester levels in CD patients as compared with the controls. There was no significant difference in the level of total short-chain carnitine esters (C2–C5 : 1) between the CD patients (17·301 (SEM 0·155) μmol/l) and the healthy controls (16·966 (SEM 0·146) μmol/l).

Table 1. Comparison of the alleles of OCTN cation transporters and NOD2/CARD15 genes in patients with Crohn's disease (*n* 100) and controls (*n* 94)

		Patients		Controls	
		n	%	*n*	%
CARD15 genotype					
R702W	CC	87	87·0	87	92·6
	CT	12	12·0	5	5·3
	TT	1	1·0	2	2·1
	T allele frequency	7·00		4·79	
G908R	GG	94	94·0	93	98·9
	GC	6	6·0	1	1·16
	CC	–		–	
	C allele frequency	3·00		0·53	
1007finsC	–	83	83·0	90	95·7
	–insC	15	15·0	4	4·3
	insC insC	2	2·0	–	
	Cins allele frequency	9·50		2·13	
SLC22A4 genotype					
C1672T	CC	37	37·0	28	29·8
	CT	54	54·0	45	47·9
	TT	9	9·0	21	22·3
	T allele frequency	36·0		46·3	
SLC22A5 genotype					
G-207C	GG	31	31·0	19	20·2
	GC	54	54·0	51	54·3
	CC	15	15·0	24	25·5
	C allele frequency	42·0		52·7	
	TC haplotype	9	9·0	19	20·2

The level of octenoylcarnitine was decreased in the healthy subjects; and the level of total medium-chain acylcarnitines (C6–C12) was 0·672 (SEM 0·007) in the CD patients *v.* 0·627 (SEM 0·007) μmol/l in the control subjects. In the long-chain acylcarnitine group the plasma levels of myristoleyl-, palmitoyl- and oleylcarnitine were significantly decreased in the healthy group. The level of total long-chain carnitine esters (C14–C18 : 1OH) was 0·612 (SEM 0·006) in the patients with CD *v.* 0·547 (SEM 0·005) μmol/l in the controls.

The level of total carnitine esters (the sum of the individual esters) was 47·255 (SEM 0·236) μmol/l in the CD patients and 48·779 (SEM 0·243) μmol/l in the controls.

The plasma carnitine ester profiles with different SLC22A4 1672 C and T genotypes are shown in Table 3 and with different SLC22A5 -207 G and C genotypes in Table 4. Albeit there were some trends for change for some individual esters (C4 and C5 carnitines), there were no statistically significant differences in the levels of any carnitine esters for any genetic variants when the heterozygous or the homozygous populations were compared. Similarly, TC haplotype did not modify the carnitine ester profile either in the CD group (Table 5) or in the controls (not shown).

Discussion

Several studies have reported an association between three CARD15 coding variants (R702W, G908R and 1007finsC) and CD in Caucasian populations, whereas these mutations have not been found at all in Asian patients (Inoue *et al.* 2002; Leong *et al.* 2003). In the present study the carriage rate for the three common CD-associated CARD15 mutations was 32 % in the patients group and 12·8 % in healthy controls.

Table 2. Plasma carnitine ester profiles (in μmol/l) in the mixed group of Crohn's disease patients (*n* 85) and controls (*n* 48)

	Patients		Controls	
	Mean	SEM	Mean	SEM
Free carnitine (C0)	28·670	0·052	30·639	0·927
Short-chain acylcarnitines				
Acetylcarnitine (C2)	16·598	0·402	16·160	0·548
Propionylcarnitine (C3)	0·243*	0·008	0·283	0·014
Butyrylcarnitine (C4)	0·274*	0·009	0·301	0·013
Isovalerylcarnitine (C5)	0·147*	0·006	0·185	0·009
Tiglylcarnitine (C5: 1)	0·039	0·002	0·037	0·002
Medium-chain acylcarnitines				
Hexanoylcarnitine (C6)	0·103	0·004	0·092	0·004
Octanoylcarnitine (C8)	0·155	0·007	0·144	0·009
Octenoylcarnitine (C8: 1)	0·086*	0·006	0·069	0·005
Decanoylcarnitine (C10)	0·160	0·008	0·152	0·009
Decenoylcarnitine (C10: 1)	0·119	0·006	0·125	0·008
Lauroylcarnitine (C12)	0·049	0·003	0·045	0·003
Long-chain acylcarnitines				
Myristoylcarnitine (C14)	0·023	0·001	0·021	0·001
Myristoleylcarnitine (C14: 1)	0·048*	0·003	0·037	0·003
Palmitoylcarnitine (C16)	0·140*	0·005	0·122	0·004
Palmitoleylcarnitine (C16: 1)	0·048	0·002	0·043	0·003
Stearoylcarnitine (C18)	0·082	0·003	0·077	0·004
Oleylcarnitine (C18: 1)	0·172*	0·006	0·156	0·008
Hydroxymyristoylcarnitine (C14OH)	0·008	0·001	0·005	0·001
Hydroxypalmitoylcarnitine (C16OH)	0·033	0·002	0·033	0·002
Hydroxypalmitoleylcarnitine (C16: 1OH)	0·038	0·001	0·037	0·002
Hydroxyoleylcarnitine (C18: 1OH)	0·020	0·001	0·016	0·001

Mean values were significantly different from those of the control group: *$P<0·05$.

Table 3. Comparison of the plasma carnitine ester profiles (in μmol/l) in Crohn's disease patients with different SLC22A4 C1672T genotypes

	Patients, CC (*n* 33)		Patients, CT (*n* 44)		Patients, TT (*n* 8)	
	Mean	SEM	Mean	SEM	Mean	SEM
Free carnitine (C0)	29·750	0·884	27·773	0·654	29·037	2·257
Short-chain acylcarnitines						
Acetylcarnitine (C2)	16·903	0·681	16·427	0·578	16·235	0·734
Propionylcarnitine (C3)	0·248	0·011	0·242	0·013	0·227	0·029
Butyrylcarnitine (C4)	0·287	0·016	0·270	0·013	0·241	0·021
Isovalerylcarnitine (C5)	0·160	0·010	0·139	0·007	0·134	0·023
Tiglylcarnitine (C5: 1)	0·036	0·002	0·041	0·003	0·042	0·007
Medium-chain acylcarnitines						
Hexanoylcarnitine (C6)	0·108	0·006	0·101	0·005	0·089	0·010
Octanoylcarnitine (C8)	0·158	0·011	0·156	0·011	0·142	0·023
Octenoylcarnitine (C8: 1)	0·077	0·010	0·090	0·008	0·100	0·018
Decanoylcarnitine (C10)	0·167	0·013	0·157	0·012	0·147	0·028
Decenoylcarnitine (C10: 1)	0·122	0·011	0·120	0·008	0·099	0·016
Lauroylcarnitine (C12)	0·051	0·005	0·046	0·004	0·050	0·011
Long-chain acylcarnitines						
Myristoylcarnitine (C14)	0·025	0·002	0·023	0·002	0·022	0·003
Myristoleylcarnitine (C14: 1)	0·049	0·005	0·047	0·003	0·046	0·010
Palmitoylcarnitine (C16)	0·144	0·007	0·135	0·007	0·154	0·019
Palmitoleylcarnitine (C16: 1)	0·052	0·004	0·044	0·003	0·058	0·008
Stearoylcarnitine (C18)	0·085	0·004	0·082	0·005	0·077	0·010
Oleylcarnitine (C18: 1)	0·190	0·008	0·163	0·010	0·178	0·021
Hydroxymyristoylcarnitine (C14OH)	0·008	0·001	0·008	0·001	0·009	0·002
Hydroxypalmitoylcarnitine (C16OH)	0·034	0·002	0·033	0·003	0·035	0·008
Hydroxypalmitoleylcarnitine (C16: 1OH)	0·035	0·002	0·038	0·002	0·043	0·005
Hydroxyoleylcarnitine (C18: 1OH)	0·022	0·003	0·018	0·002	0·023	0·004

Table 4. Comparison of the plasma carnitine ester profiles (in μmol/l) in Crohn's disease patients with different SLC22A5 G-207C genotypes

	Patients, GG (*n* 28)		Patients, GC (*n* 45)		Patients, CC (*n* 12)	
	Mean	SEM	Mean	SEM	Mean	SEM
Free carnitine (C0)	29·835	0·972	27·686	0·613	29·559	1·834
Short-chain acylcarnitines						
Acetylcarnitine (C2)	16·297	0·677	16·406	0·522	17·929	1·335
Propionylcarnitine (C3)	0·248	0·011	0·242	0·014	0·236	0·020
Butyrylcarnitine (C4)	0·292	0·017	0·264	0·013	0·267	0·020
Isovalerylcarnitine (C5)	0·158	0·010	0·143	0·008	0·133	0·016
Tiglylcarnitine (C5: 1)	0·036	0·002	0·040	0·003	0·043	0·005
Medium-chain acylcarnitines						
Hexanoylcarnitine (C6)	0·101	0·006	0·103	0·005	0·104	0·012
Octanoylcarnitine (C8)	0·146	0·010	0·162	0·011	0·153	0·021
Octenoylcarnitine (C8: 1)	0·077	0·011	0·091	0·008	0·085	0·015
Decanoylcarnitine (C10)	0·153	0·012	0·165	0·013	0·156	0·022
Decenoylcarnitine (C10: 1)	0·114	0·011	0·123	0·009	0·111	0·014
Lauroylcarnitine (C12)	0·046	0·004	0·050	0·004	0·048	0·007
Long-chain acylcarnitines						
Myristoylcarnitine (C14)	0·024	0·002	0·024	0·002	0·022	0·002
Myristoleylcarnitine (C14: 1)	0·044	0·005	0·050	0·004	0·047	0·006
Palmitoylcarnitine (C16)	0·143	0·009	0·139	0·007	0·140	0·014
Palmitoleylcarnitine (C16: 1)	0·050	0·004	0·046	0·003	0·055	0·006
Stearoylcarnitine (C18)	0·084	0·004	0·081	0·005	0·083	0·009
Oleylcarnitine (C18: 1)	0·190	0·009	0·157	0·008	0·209	0·026
Hydroxymyristoylcarnitine (C14OH)	0·007	0·001	0·008	0·001	0·009	0·002
Hydroxypalmitoylcarnitine (C16OH)	0·034	0·003	0·034	0·002	0·031	0·005
Hydroxypalmitoleylcarnitine (C16: 1OH)	0·035	0·002	0·037	0·002	0·046	0·005
Hydroxyoleylcarnitine (C18: 1OH)	0·022	0·003	0·018	0·002	0·022	0·003

Table 5. Comparison of the plasma carnitine ester profiles (μmol/l) in Crohn's disease patients with different SLC22A4 C1672T and SLC22A5 G-207C genotypes

	Patients with TC haplotype (*n* 8)		Patients with other haplotype (*n* 77)	
	Mean	SEM	Mean	SEM
Free carnitine (C0)	29·037	2·257	28·632	0·497
Short-chain acylcarnitines				
Acetylcarnitine (C2)	16·235	0·734	16·638	0·404
Propionylcarnitine (C3)	0·227	0·029	0·245	0·008
Butyrylcarnitine (C4)	0·241	0·021	0·277	0·009
Isovalerylcarnitine (C5)	0·134	0·023	0·148	0·006
Tiglylcarnitine (C5: 1)	0·042	0·007	0·039	0·002
Medium-chain acylcarnitines				
Hexanoylcarnitine (C6)	0·089	0·010	0·104	0·004
Octanoylcarnitine (C8)	0·142	0·023	0·157	0·007
Octenoylcarnitine (C8: 1)	0·100	0·018	0·084	0·006
Decanoylcarnitine (C10)	0·147	0·028	0·161	0·008
Decenoylcarnitine (C10: 1)	0·099	0·016	0·121	0·006
Lauroylcarnitine (C12)	0·050	0·011	0·048	0·003
Long-chain acylcarnitines				
Myristoylcarnitine (C14)	0·022	0·003	0·024	0·001
Myristoleylcarnitine (C14: 1)	0·046	0·010	0·048	0·003
Palmitoylcarnitine (C16)	0·154	0·019	0·139	0·005
Palmitoleylcarnitine (C16: 1)	0·058	0·008	0·047	0·002
Stearoylcarnitine (C18)	0·077	0·010	0·083	0·003
Oleylcarnitine (C18: 1)	0·178	0·021	0·175	0·006
Hydroxymyristoylcarnitine (C14OH)	0·009	0·002	0·008	0·001
Hydroxypalmitoylcarnitine (C16OH)	0·035	0·008	0·033	0·002
Hydroxypalmitoleylcarnitine (C16: 1OH)	0·043	0·005	0·037	0·001
Hydroxyoleylcarnitine (C18: 1OH)	0·023	0·004	0·019	0·001

The results are in concordance with previously reported rates of 30–50 % in CD and 7–20 % in controls from other European CD populations (Ahmad *et al.* 2002; Lesage *et al.* 2002; Vermeire *et al.* 2002; Brant *et al.* 2003; Mendoza *et al.* 2003; Arnott *et al.* 2004; Vind *et al.* 2005). The allele frequencies of R702W, G908R and 1007finsC mutations we have found (7·0, 3·0 and 9·5 % in patients and 4·8, 0·5 and 2·1 % in controls, respectively) are in agreement with other studies from Europe (6·7–12·9, 3·3–6·1 and 6·6–16 % in CD and 3·5–6·9, 0·6–3·0 and 1·0–4·4 % in controls) (Lesage *et al.* 2002; Vermeire *et al.* 2002; Mendoza *et al.* 2003; Arnott *et al.* 2004).

By resequencing five genes in the IBD5 interval, Peltekova *et al.* (2004) identified two novel polymorphisms in the SLC22A4 and SLC22A5 genes. These mutations (SLC22A4 C1672T and SLC22A5 G-207C) appeared to form a two-allele risk haplotype (OCTN-TC) for development of CD and showed significant interactions with CD-associated CARD15 mutations (Peltekova *et al.* 2004). This observation has been confirmed in some studies (Newman *et al.* 2005; Noble *et al.* 2005; Torok *et al.* 2005), but no association was found in others (Yamazaki *et al.* 2004; Noble *et al.* 2005; Torok *et al.* 2005; Vermeire *et al.* 2005). In the present study we investigated a larger group of Hungarian patients, first in the literature, and could not detect the accumulation of either SLC22A4 and SLC22A5 susceptibility variants or the TC haplotype in the CD patients; thereby, the present findings do not support the susceptibility role of these haplotypes in the development of CD in this population.

The primary biochemical function of carnitine is its involvement in the energy production of cells (Bieber, 1988). Moreover, via ester formation it facilitates the removal of SCFA and medium-chain fatty acids, which accumulate as a result of normal and abnormal metabolism in the mitochondria. The circulating carnitine ester spectrum can reflect affected cellular metabolism of the SCFA, medium- and long-chain fatty acids (Rashed, 2001).

Very limited data are available on the carnitine status in CD; these studies are from the enzymatic radiochemical carnitine determination era. Using this approach, Demirkol *et al.* (1994) found a significant decrease of free and total carnitine concentrations in paediatric patients with CD. In another study a dramatically decreased free carnitine and significantly increased long- and short-chain acylcarnitine concentrations were observed, resulting in a net increase in total carnitine. However, in that study patients with different types of inflammatory disorders after multiple trauma or head injury were examined (Adlouni *et al.* 1988). Therefore, these data are not really comparable to the findings of the present study of a mixed population of CD-affected patients. The data presented here show a complex change of the carnitine esters, and the current knowledge is not enough for the full understanding of the findings revealed.

Proinflammatory cytokines are essential in the pathogenesis of CD. Activation of NF-κB, which plays a central role in immune and inflammatory responses and is involved in the proinflammatory cytokine gene transcription, is increased in the intestinal mucosa of CD patients (Schreiber *et al.* 1998). Butyrate inhibits the translocation of NF-κB transcription factor to the nucleus, thereby preventing the activation of proinflammatory genes (Segain *et al.* 2000; Di Sabatino *et al.* 2005). Thus, the observed butyrylcarnitine level can be an element of the development of inflammatory processes. However, another explanation cannot be ruled out.

The OCTN1 and OCTN2 play an essential role in the plasmalemmal carnitine uptake, thereby regulating the normal tissue/circulating carnitine balance (Tamai *et al.* 1998; Wu *et al.* 1998, 2000). In fibroblasts with C1672T missense substitution in exon 9 of the SLC22A4 gene resulting in marked changes in OCTN1 transporter activity, and also in fibroblasts with G-207C transversion in the SLC22A5 promoter region causing OCTN2 promoter function impairment, the carnitine uptake was affected showing that these genetic variations can associate with functional consequences (Peltekova *et al.* 2004). The authors suggested that these genes had a role in chronic inflammatory disorders, moreover, they supposed that these gene variants may cause disease by impairing OCTN activity or expression, reducing carnitine transport in a cell-type and disease-specific manner. Here we analysed the plasma carnitine ester profiles of the patients according to the different SLC22A4 C1672T and SLC22A5 G-207C genotypes. To our surprise, the variations of the genotypes were not associated with influenced carnitine ester profiles. In the current study we could exclude therefore any major effects of the genotype on the carnitine esters.

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