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Impaired portal circulation resulting from L-arginine deficiency in patients with lysinuric protein intolerance

Lysinuric protein intolerance (LPI) is a rare autosomal recessive defect of the dibasic amino acid transporter, which is caused by mutations in the *SLC7A7* gene and results in L-arginine deficiency.¹ L-arginine is the only known substrate for nitric oxide (NO) production,² and thus the availability of L-arginine is crucial for regulation of NO production.³ NO is an endothelium derived vascular relaxation factor that plays an important role in vascular endothelial function.^{4–6} However, the effects of L-arginine deficiency on portal circulation in humans have not been studied.

Portal circulation was evaluated in seven Japanese LPI patients (six males). Seven healthy subjects (six males) without liver disease served as controls. The study was approved by the ethics committee of our university. Mean age of the LPI patients was 18.9 (10.7) years (range 5–32) and that of the controls 15.6 (7.3) years (range 7–26). Mean body weight of the patients was 38.0 (11.4) kg (range 14.6–50.3) and that of the controls 42.1 (13.6) kg (range 19.0–58.0). There was no significant difference in age or body weight between the two groups. Also, there was no significant difference in body mass index between the two groups (LPI patients 18.7 (1.9) kg/m² (range 16.4–21.2); controls 17.4 (2.0) kg/m² (range 13.2–18.9)).

Six patients were homozygotes of a common mutation in the *SLC7A7* gene (R410X) and one was a compound heterozygote with two mutations (S238F and R410X).^{7,8} In all LPI patients, plasma levels of L-arginine (38.5 (8.1) nmol/ml (range 22.9–47.6)) were lower than the lower limit of the normal range (54–130 nmol/ml), and plasma levels of L-lysine and L-ornithine were also less than the normal range, except for one patient.

Portal blood flow volume (PBFV) and portal vein diameter (PVD) were determined by ultrasonography.^{9,10} Serum levels of NO derivatives and PBFV before and after administration of L-arginine or an NO donor, isosorbide dinitrate (ISDN), were evaluated. Fasting serum levels of NO derivatives in patients (67.3 (8.9) μ mol/l) were significantly lower than those in controls (120.3 (19.2) μ mol/l) ($p < 0.01$) (fig 1A). PBFV in patients (411.3 (173.1) ml/min) was significantly lower than in controls (837.5 (214.2) ml/min) ($p < 0.01$) (fig 1B). PVD in patients (6.1 (1.4) mm) was also significantly lower than in controls (8.5 (1.3) mm) ($p < 0.01$) (fig 1C). Serum NO levels increased from baseline in the three patients given ISDN and in the three patients given L-arginine: from 71.8 (8.9) to 103.5

(6.2) μ mol/l in patients receiving ISDN, and from 64.5 (10.3) to 95.6 (9.1) μ mol/l in patients receiving L-arginine. Serum NO levels after administration of ISDN or L-arginine to patients were almost restored to those in controls. PBFV increased from baseline in the three patients given ISDN and in the three patients given L-arginine: from

301.3 (96.8) to 687.6 (111.8) ml/min in patients receiving ISDN and from 521.3 (170.9) to 1119.3 (192.6) ml/min in patients receiving L-arginine. PBFV after administration of ISDN or L-arginine was restored to close to that in controls (fig 1D, E).

It is still not known whether L-arginine administration can improve portal circulation

Figure 1 Serum levels of nitric oxide (NO) derivatives (nitrite and nitrate) were measured with an NO₂/NO₃ Assay Kit-Fil (Wako Pure Chemical Industries, Osaka, Japan). Isosorbide dinitrate (5 mg/5 kg up to a maximum of 40 mg) was administered to three patients transdermally for three hours, and L-arginine hydrochloride (0.5 g/kg) was infused to three other patients intravenously for one hour. Statistical analysis was performed using the Mann-Whitney U test or Wilcoxon signed rank test. (A) Fasting concentrations of serum NO derivatives in patients with lysinuric protein intolerance (LPI) and controls. Baseline data for portal vein flow in LPI patients and controls. Baseline portal blood flow volume (PBFV) (B) and baseline portal vein diameter (PVD) (C). Changes in PBFV in LPI patients before and after isosorbide dinitrate (D) or L-arginine (E) administration. The shaded area represents the mean (SD) of the PBFV in controls. Three LPI patients were given isosorbide dinitrate (D) and three were given L-arginine (E).

by restoring endothelial cell function of the portal vein in patients. However, the present study suggests that L-arginine deficiency in LPI can cause impairment of portal circulation via a decrease in NO production.

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Association study of *TNFSF15* polymorphisms in Japanese patients with inflammatory bowel disease

Tumour necrosis factor superfamily (TNFSF) 15 is a novel member of the TNFSF and its mRNA and protein expression is upregulated in inflammatory bowel disease (IBD), particularly in Crohn's disease (CD).^{1,2} Recently, Yamazaki *et al* performed a large scale case control study using single nucleotide polymorphism (SNP) markers and reported that polymorphisms in *TNFSF15* conferred susceptibility to CD in both Japanese and UK populations.³ They also suggested a potential association between a Caucasian ulcerative colitis (UC) cohort and *TNFSF15*, but this association was not studied in Japanese patients. To investigate this possible association between *TNFSF15* and Japanese UC, and to replicate this association with CD in Japanese, we performed a case control association study in Japanese patients with CD and UC.

We selected six SNPs—*tnfsf15*₂₆, *tnfsf15*₂₈, *tnfsf15*₃₁, *tnfsf15*₃₃, *tnfsf15*₃₅, *tnfsf15*₃₆—which were reported to show a strong association ($p < 10^{-10}$), and genotyped these six SNPs in 286 patients with CD, 263 patients with UC, and 277 healthy controls (HCs) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. All patients and HCs were Japanese, and none had a family history of IBD.

We replicated all six SNPs that were significantly associated with the Japanese CD cohort (table 1). On the other hand, in contrast with Caucasians,³ none of the SNPs were associated with the Japanese UC cohort. Risk allele frequencies of *tnfsf15*₃₅, *tnfsf15*₃₆, and *tnfsf15*₃₁ were higher in the CD group with anal lesions than in those without, but the associations were statistically weak ($p = 0.019$, 0.019 , and 0.037 , respectively). No significant differences were found in allele frequencies between the CD subgroups classified by age at diagnosis, location of disease, existence of fistula, stenosis, need for steroid therapy, and past history of surgical treatment, and also the UC subgroups classified by age at diagnosis, extent of disease, need for intensive intravenous steroid therapy, and need for surgical treatment.

In this study, we confirmed the findings of a previous report concerning a significant association between *TNFSF15* and CD. On the other hand, no evidence for an association with Japanese UC was observed, although a potential association with Caucasian UC was reported. It is generally accepted that UC and CD may share some susceptibility genes. Ethnic differences in genetic susceptibility may be explained by differences in the haplotypic background. Some *TNFSF15* polymorphisms identified in the Japanese were monomorphic or nearly monomorphic in the Caucasian population.³ Thus it seems likely that population specific patterns of haplotypes may contribute to differences in UC susceptibility.

Although a previous report described that *tnfsf15*₂₈ showed the lowest p value and highest odds ratio among the SNPs in *TNFSF15*,³ our results showed that p values and odds ratios of *tnfsf15*₃₆ and *tnfsf15*₃₅ were similar to those of *tnfsf15*₂₈. Thus to identify the pathogenic SNP, a functional study is clearly needed. We analysed the transcription factor binding sites in the promoter region of *TNFSF15* by TFSEARCH⁴ and found that GATA-1, 2, and 3 possibly bind the *tnfsf15*₃₅-T allele while the GATA binding *cis* element is absent in the *tnfsf15*₃₅-C allele (risk associated). It is well known that GATA-3 promotes a Th2 mediated immunological state and suppresses expression of Th1 mediated cytokines.^{5,6} These findings have raised the possibility that GATA-3 may not bind the promoter region with the *tnfsf15*₃₅-C risk allele resulting in lack of suppression of *TNFSF15* expression. Consequently, over-expressed *TNFSF15* promotes a Th1 mediated immunological state and initiates or exacerbates the severity of CD. Although we do not have experimental evidence for this hypothesis, we intend to elucidate the functional significance in the future.

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Table 1 Allele frequencies of *TNFSF15*

SNP	dbSNP	Location	Allele1	Allele2	Frequency of allele2 (%) / OR / p value		
					HCS (n=277)	CD (n=286)	UC (n=263)
<i>tnfsf15</i> ₃₆	rs7848647	5'-flanking region	G	A	39.0	25.7 (OR = 1.85, $p = 1.84 \times 10^{-6}$)	36.3 (NS)
<i>tnfsf15</i> ₃₅	rs6478109	5'-flanking region	C	T	39.0	25.7 (OR = 1.85, $p = 1.84 \times 10^{-6}$)	36.3 (NS)
<i>tnfsf15</i> ₃₃	rs6478108	Intron 1	A	G	51.8	37.6 (OR = 1.78, $p = 1.60 \times 10^{-6}$)	52.3 (NS)
<i>tnfsf15</i> ₃₁	rs4979462	Intron 1	A	G	39.5	26.2 (OR = 1.84, $p = 1.98 \times 10^{-6}$)	36.5 (NS)
<i>tnfsf15</i> ₂₈	—	Intron 3	C	T	53.1	38.8 (OR = 1.78, $p = 1.58 \times 10^{-6}$)	52.3 (NS)
<i>tnfsf15</i> ₂₆	rs3810936	Exon 4 (Val201Val)	G	A	40.3	26.9 (OR = 1.83, $p = 2.15 \times 10^{-6}$)	36.3 (NS)

SNP, single nucleotide polymorphism; HCs, healthy controls; CD, Crohn's disease; UC, ulcerative colitis; NS, no significant. OR, odds ratio.