Table 2

Demographic and lipid profile of all the participants according to genotype

CETP D442G (rs2303790)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47	91.6	1.53 (0.001)	1.37 (0.025)	3.06 (0.021)
hetero	48.4	8.1	1.75 (0.004)	1.15 (0.061)	2.90 (0.075)
homo	46.5	0.2	1.81 (0.18)	1.60 (0.101)	3.19 (1.580)
			p=0.000	p=0.071	p=0.154

CETP Int14 +1 G → A (rs5742907)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47	99.4	1.54 (0.009)	1.36 (0.024)	3.06 (0.020)
hetero	58.7	0.6	2.12 (0.262)	1.72 (0.362)	3.08 (0.316)
			p=0.000	p=0.241	p=0.938

CETP TaqIB (rs708272)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	46.8	35.8	1.50 (0.016)	1.36 (0.036)	3.00 (0.033)
B1B2	48.4	48.4	1.54 (0.013)	1.38 (0.038)	3.08 (0.030)
B2B2	48.2	15.8	1.66 (0.024)	1.25 (0.043)	3.08 (0.051)
			p=0.000	p=0.160	p=0.362

LPL S447X (rs328)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	47.3	78	1.53 (0.011)	1.37 (0.029)	3.06 (0.023)
hetero	46.2	20.7	1.60 (0.020)	1.24 (0.043)	3.06 (0.046)
homo	48	1.3	1.63 (0.101)	1.08 (0.125)	3.29 (0.189)
			p=0.004	p=0.032	p=0.487

LIPC 514CT (rs1800588)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	49.7	24.9	1.49 (0.018)	1.37 (0.046)	3.11 (0.040)
CT	45.6	50.4	1.53 (0.013)	1.33 (0.034)	3.03 (0.029)
TT	47.6	24.7	1.63 (0.020)	1.39 (0.050)	3.06 (0.040)
			p=0.000	p=0.520	p=0.255

APOC3 SstI (rs5128)

genotype	age	%	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	46.6	42	1.56 (0.015)	1.32 (0.039)	3.06 (0.032)
S1S2	47	45.8	1.54 (0.013)	1.34 (0.033)	3.03 (0.029)
S2S2	48.9	12.2	1.52 (0.025)	1.53 (0.070)	3.11 (0.060)
			p=0.413	p=0.021	p=0.434

Data are expressed as mean (SEM). P-value was based on analysis of covariance.

Table 3

Demographic and lipid profile of male participants according to genotype

CETP D442G (rs2303790)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	351	1.36 (0.020)	1.60 (0.052)	3.11 (0.045)
hetero	26	1.60 (0.105)	1.19 (0.176)	2.98 (0.194)
		p=0.003	p=0.035	p=0.453

CETP TaqIB (rs708272)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	121	1.33 (0.034)	1.64 (0.087)	3.06 (0.073)
B1B2	203	1.36 (0.026)	1.55 (0.068)	3.11 (0.064)
B2B2	53	1.56 (0.063)	1.53 (0.147)	3.13 (0.107)
		p=0.001	p=0.664	p=0.758

LPL S447X (rs328)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	292	1.36 (0.022)	1.65 (0.060)	3.08 (0.047)
hetero	81	1.43 (0.048)	1.36 (0.082)	3.16 (0.112)
homo	4	1.51 (0.386)	0.95 (0.295)	2.80 (0.513)
		p=0.278	p=0.029	p=0.617

LIPC 514CT (rs1800588)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	99	1.32 (0.032)	1.66 (0.094)	3.08 (0.072)
CT	188	1.40 (0.032)	1.51 (0.075)	3.08 (0.069)
TT	90	1.40 (0.041)	1.60 (0.095)	3.08 (0.085)
		p=0.266	p=0.499	p=0.996

APOC3 SstI (rs5128)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	165	1.37 (0.031)	1.50 (0.073)	3.16 (0.072)
S1S2	173	1.40 (0.031)	1.58 (0.076)	3.00 (0.060)
S2S2	39	1.31 (0.054)	1.92 (0.162)	3.13 (0.138)
		p=0.473	p=0.041	p=0.196

Data are expressed as mean (SEM). P-value was based on analysis of covariance.

Table 4

Demographic and lipid profile of female participants according to genotype

CETP D442G (rs2303790)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	440	1.58(0.018)	1.128 (0.0412)	2.93 (0.041)
hetero	34	1.67 (0.074)	1.15 (0.092)	2.98 (0.140)
		p=0.002	p=0.590	p=0.306

CETP TaqIB (rs708272)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
B1B1	183	1.58 (0.028)	1.13 (0.057)	2.93 (0.062)
B1B2	220	1.67 (0.026)	1.15 (0.066)	2.98 (0.059)
B2B2	72	1.75 (0.043)	0.92 (0.057)	2.85 (0.105)
		p=0.004	p=0.127	p=0.461

LPL S447X (rs328)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
wt	369	1.62 (0.020)	1.14 (0.046)	2.95 (0.046)
hetero	102	1.73 (0.038)	0.99 (0.065)	2.85 (0.081)
homo	4	1.97 (0.164)	0.72 (0.177)	3.89 (0.321)
		p=0.010	p=0.185	p=0.054

LIPC 514CT (rs1800588)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
CC	102	1.59 (0.041)	1.15 (0.089)	2.93 (0.086)
CT	249	1.63 (0.022)	1.04 (0.046)	2.90 (0.050)
TT	124	1.73 (0.037)	1.20 (0.091)	3.03 (0.090)
		p=0.014	p=0.210	p=0.406

APOC3 SstI (rs5128)

genotype	n	HDL-c (mmol/l)	TG (mmol/l)	LDL-c (mmo/l)
S1S1	207	1.65 (0.028)	1.05 (0.054)	2.90 (0.062)
S1S2	208	1.62 (0.026)	1.18 (0.067)	2.93 (0.059)
S2S2	60	1.75 (0.045)	1.08 (0.079)	3.03 (0.106)
		p=0.078	p=0.272	p=0.608

Data are expressed as mean (SEM). P-value was based on analysis of covariance.

Table 5.

Incidence of *CETP* TaqIB, D442G, and *LPL* S447X genetypes according to HDL levels.

CETP TaqIB	HDL-c (mmol/l)			
genotype	1.0> (8.3%)	1.0_, 2.58> (89.9%)	2.58_ (1.8%)	
B1B1	72 (9.9%)	644 (88.8%)	9 (1.2%)	p=0.009
B1B2	79 (8.2%)	870 (90.2%)	16 (1.7%)	
B2B2	15 (4.8%)	284 (91.6%)	11 (3.5%)	
CETP D442G				
WT	161 (8.7%)	1671 (89.8%)	29 (1.6%)	p=0.011
Hetero	5(3.6%)	125 (91.2%)	7 (5.1%)	
Homo	0 (0%)	2 (100%)	0 (0%)	
LPL S447X				
WT	134 (8.9%)	1354 (89.4%)	26 (1.7%)	p=0.002
Hetero	21 (5.0%)	390 (93.3%)	7 (1.7%)	
Homo	2 (8.0%)	21 (84.0%)	2 (8.0%)	

Column percentage is shown on top. Each box shows the number of participants in each category and its percentage in each genotype.

Supplemental table

		Age	T-cho (mmol/l)	TG (mmol/l)	HDL-c (mmol/l)	LDL-c (mmol/l)
total	mean	45.9	5.15	1.33	1.52	3.03
n=12,839	median	48.0	5.10	1.06	1.49	2.97
this study	mean	47.1	5.18	1.35	1.53	3.02
n=2,267	median	48.0	5.10	1.06	1.49	2.92

J Hum Genet (2004) 49:24–28
DOI 10.1007/s10038-003-0101-8

ORIGINAL ARTICLE

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Hypercholesterolemia associated with splice-junction variation of inter-α-trypsin inhibitor heavy chain 4 (*ITIH4*) gene

Received: 20 August 2003 / Accepted: 15 October 2003 / Published online: 6 December 2003

Abstract Factors predisposing to the phenotypic features of higher total cholesterol (T-Cho) have not been clearly defined. Here we report an association between a C/T single nucleotide polymorphism at IVS17+8 in the inter-alpha-trypsin inhibitor heavy chain 4 gene (*ITIH4*) and plasma total cholesterol levels in 351 adult individuals from an east-central area of Japan. Age and gender-adjusted levels of plasma T-Cho, LDL-cholesterol, triglyceride, and HDL-cholesterol were analyzed. When we separate the subjects into two genotypic groups regarding this single nucleotide polymorphism (SNP), those who lack the T-allele had significantly higher plasma T-Cho levels than the others who bear T-allele (mean 252.3 mg/dl versus 241.7 mg/dl; $p=0.009$). Of the 309 individuals without the T-allele, approximately 90% presented with hypercholesterolemia, whereas only 10% were hypercholesterolemic among 42 individuals with the T-allele ($p<0.0001$). These data suggest that genetic variation at *ITIH4* locus is one of the likely candidate determinants for plasma cholesterol metabolisms.

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Keywords Inter-alpha-trypsin inhibitor heavy chain 4 · Plasma total cholesterol (T-Cho) · Single nucleotide polymorphism (SNP) · Modifier gene

Introduction

Accumulating evidences derived from clinical, epidemiological, and experimental studies suggest that lipid and lipoprotein concentrations in plasma reflect the influence of complex genetic loci, at least in part (Zannis and Breslow 1985); defects are known only for some classical types of hyperlipoproteinemia that affect members of families following Mendelian traits. However, the genetic mechanisms responsible for most types of familial dyslipoproteinemia appear to be complex, not monogenic.

Hyperlipoproteinemia is one of the most important predictors of cardiovascular diseases determined by genetic risk factors as well as environmental factors (Hegele 2001; Cullen et al. 1997). Clarification of the genetic risk factors is essential for diagnosis, prevention, and early effective treatment of hyperlipidemias. In addition, if the relevant genes were identified, the pathogenesis of these diseases would be explained by the variation of those genes or adjacent genes. Several gene polymorphisms have previously been associated with plasma lipoprotein abnormalities (reviewed in Goldstein et al. 1995). However, those are only a part of the all determinants, obviously.

ITIH4 is a glycoprotein mainly expressed in the liver tissue (Nishimura H et al. 1995). It belongs to the inter-α-trypsin inhibitor family of serine protease inhibitors (ITIH1, 2, 3, and 4), which have diverse functions as antiapoptotic and matrix-stabilizing molecules important throughout the development. In addition, *ITIH4* gene locates in chromosomal region 3p21.1-p22 were recently described as one of the candidate quantitative trait locus (QTL) for dyslipidemias (Yuan et al. 2000). Expression in the liver, the role as a protease inhibitor,

and the location in the candidate QTL region constituted rationales for selecting this gene as a candidate to study the correlation between genetic variation and lipoprotein profile.

In the course of serial investigations of population genetics for hyperlipoproteinemia in a cohort of an area located in east-central Japan, we recognized a correlation between the plasma total cholesterol (T-Cho) levels and a variation of the inter-alpha-trypsin inhibitor heavy chain 4 (*ITIH4*) gene. Here we focused on the analysis of the potential effect of genetic variation in *ITIH4*, investigating the correlation of plasma lipoprotein profile with the genetic variation of this gene.

Subjects and methods

Subjects

Subjects were obtained from participants of a cohort study that was originally carried out concurrently with health-check screening in an area in east-central Japan, as described in detail previously (Fujita et al. 2003). In brief, 22,228 participants were initially screened, and hypercholesterolemic individuals whose LDL-cholesterol (LDL-C) levels were higher than 140 mg/dl (LDL-C ≥140 mg/dl) were selected. To the present study, 351 individuals were recruited who gave their written informed consent prior to the study, which was approved by the Institutional Review Boards of the Research Consortium. Physical and clinical profiles of these subjects (ages, male to female ratios, body mass indices, and plasma lipoprotein and lipid levels) are indicated in Table 1. None of the selected participants had medical complications or was undergoing treatment for conditions known to affect plasma lipoprotein levels, such as pituitary disease, hypothyroidism or hyperthyroidism, diabetes mellitus, liver disease, and renal disease. None was receiving antihyperlipidemic therapy. The selected participants visited lipid clinics for detailed examination of their lipoprotein profile and other clinical parameters. Blood was collected after 12–16 h of fasting. Genomic DNA was extracted, as previously described (Yoshida et al. 2002).

Table 1 Physical and clinical profiles of the subjects. *NS* not significant, *M/F* male/female, *BMI* body mass index, *TC* total cholesterol, *LDL-C* LDL-cholesterol, *HDL-C* HDL-cholesterol, *TG* triglyceride

	T-allele (+)	T-allele (−)	*p* value
Number	42	309	–
Gender (M/F)	15/27	140/169	NS
Ages (years)	60.0 ± 8.6	61.4 ± 9.1	NS
BMI (kg/m^2)	23.8 ± 3.2	23.8 ± 3.8	NS
TC (mg/dl)	241.7 ± 23.8	252.3 ± 23.5	0.009
LDL-C (mg/dl)	168.8 ± 22.0	169.7 ± 20.3	NS
HDL-C (mg/dl)	46.9 ± 10.9	51.5 ± 12.6	0.014
TG (mg/dl)	146.7 ± 77.1	160.4 ± 89.6	NS

The *p* value was calculated by Mann-Whitney test, except for gender; $\kappa 2$ test was conducted for distribution analysis of gender Values are expressed in mean ± SD

Measurement of lipoproteins

Lipid and lipoprotein concentrations were measured by procedures described previously (Hattori et al. 2002); i.e., plasma cholesterol and triglyceride concentrations were assayed enzymatically, and concentrations of HDL-cholesterol (HDL-C) were determined by the $MgCl_2$-dextran precipitation method. LDL-C concentration was calculated by subtracting HDL-C level from the fraction of both LDL-C and HDL-C, as described elsewhere (Ishii et al. 2002).

Selection criteria of single nucleotide polymorphisms (SNPs)

We focused on all SNPs that either accompanies amino acid substitution, SNPs adjacent to the exon/intron junction, and SNPs that locate in the putative promoter region in the entire *ITIH4* gene. The complete list of such SNPs in Table 2 describes nucleotide variation, amino acid variation, location, reference database (JSNP and dbSNP), allele frequency, and heterozygosity in the population. We examined SNPs whose percent-heterozygosity exceeded 10% to obtain substantial statistic analytical power. Thus, out of the six SNPs listed in Table 2, IVS17+8C/T and IVS20+8A/G were selected for the present study.

Genotyping for SNP in the *ITIH4* gene

PCR amplification of the flanking sequence for the polymorphism IVS17+8C/T and IVS20+8A/G at the *ITIH4* locus was performed using a condition described previously (Iida et al. 2002). The surrounding sequence of the SNP as well as the primer sequences and the experimental condition were obtained from the published reports (Haga et al. 2002). SNP genotyping was performed by Invader assay (Third Wave Technologies. Madison, WI, USA) (Saito et al. 2002) using PCR products of the flanking sequence and the probes designed and synthesized by the supplier (Ohnishi et al. 2001).

Statistical analysis

Plasma levels of lipoproteins were adjusted by gender and ages of the subjects using standard data obtained from 11,994 individuals of a 2001 cohort study of the general Japanese population. Coefficients of skewness and kurtosis were calculated to test deviation from a normal distribution. Because the clinical and biochemical

Table 2 Summary of the selected polymorphisms in the ITIH4 gene. *SNP* single nucleotide polymorphism

No.	SNP	nt.	Location	JSNP-ID[a]	dbSNP[b]	Allele frequency (heterozygosity)[c]
1	L669Q	T/A	Exon17	JMS-JST073530	rs2276814	0.997:0.003 (0.7%)
2	IVS17+8C/T	C/T	Intron17	JMS-JST182668	rs3821831	0.933:0.067 (12.5%)
3	P698T	C/A	Exon18	–	rs4687657	ND (ND)
4	I714M	C/G	Exon18	JMS-JST073528	rs2256734	0.997:0.003 (0.7%)
5	IVS20+8A/G	A/G	Intron20	JMS-JST073526	rs2245538	0.566:0.434 (49.1%)
6	L791P	T/C	Exon21	–	rs2535621	ND (ND)

[a] Number from Japanese SNP database (http://snp.ims.u-tokyo.ac.jp/index_ja.html)
[b] Number from dbSNP database of NCBI (http://www.ncbi.nlm.nih.gov/SNP/)
[c] Data from dbSNP database or from JSNP database

traits in each genotypic group did not always distribute normally, we applied a nonparametric Mann-Whitney test or an analysis of variance (ANOVA) with linear regression analysis as a post hoc test ($p < 0.05$) to compare those traits among groups divided by a single SNP (Fujiwara et al. 2002). Fisher's exact test was used to compare differences in the prevalence of hypercholesterolemia among the population. Chi-square tests were invoked to detect Hardy-Weinberg equilibrium.

Results

To carry out a correlation analysis of the potential effect of SNPs, IVS17+8C/T and IVS20+8A/G, 351 individuals were selected from participants for a cohort of an area initially screened by criteria that defined individuals harboring hyperlipidemic risks. Because of initial screening for the health-check assessment, basal level of each value was a little higher than that of data from the general Japanese population. However, the average differences were less than 0.5×SD in general. When the subjects were genotypically categorized into three groups for each SNP (for instance, three homozygous minor T-allele carriers, 39 heterozygous carriers and 309 homozygous C-allele carriers, for IVS17+8C/T), no deviation of genotype frequencies from Hardy-Weinberg equilibrium was observed ($p = 0.38$, χ^2-test).

Distribution and mean values of T-Cho, TG, LDL-C and HDL-C were analyzed among the genotypically divided groups by each SNP, and significant difference in T-Cho and HDL-C was detected among the three groups divided by IVS17+8C/T genotypes. Plasma T-Cho levels of homozygous minor T-allele carriers ($n = 3$), heterozygous carriers ($n = 39$), and homozygous C-allele carriers ($n = 309$) were 200.4±39.7 mg/dl, 244.9±19.5 mg/dl, and 252.3±23.5 mg/dl respectively, indicating a codominant T-Cho lowering effect of the minor T-allele ($r = 0.18$, $p = 0.0006$). Similarly, plasma HDL-C levels were 31.1±6.9 mg/dl, 48.1±10.2 mg/dl, and 51.5±12.6 mg/dl, respectively, indicating a codominant HDL-C lowering effect of the minor T-allele ($r = 0.15$, $p = 0.006$). No statistically significant difference was detected in the plasma lipoprotein levels among the groups categorized by IVS20+8A/G, suggesting presence of a break of haplotype blocks within the gene (data not shown).

We repeatedly evaluated the genotypic effect by categorization of the subjects into two groups, those who lack the T-allele (C/C) and those who bear at least one T-allele (C/T and T/T), since the homozygous subjects carrying the minor allele was rare ($n = 3$). By this categorization, the former subjects had significantly higher plasma T-Cho levels than the latter (mean±SD 252.3±23.5 mg/dl versus 241.7±23.8 mg/dl; $p = 0.009$) (Fig. 1A). Similarly, the former subjects had significantly higher HDL-C levels than the latter (mean±SD 51.5±12.6 mg/dl versus 46.9±10.9 mg/dl; $p = 0.014$).

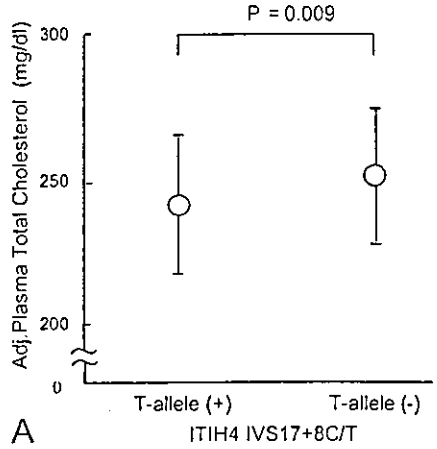
Since the data presented here suggested an involvement of *ITIH4* locus in the expression of the hypercholesterolemic phenotype in these populations, we correlated the manifestation of the hypercholesterolemia with respect to presence or absence of T-alleles of *ITIH4* IVS17+8C/T. Hypercholesterolemia was defined as plasma cholesterol level above the reference values after age and gender adjustment (T-Cho >220 mg/dl). This criterion is based on a recommended preclinical level of hyperlipidemic individuals who have to be alerted for high risk for ischemic heart disease. It classified 291 subjects as having hypercholesterolemia among the study population consisting of 351 individuals. Of the 309 individuals lacking the T-allele, 286 presented with hypercholesterolemia (93%) whereas only five did so among 42 individuals with T-allele (12%). Distribution difference was significant when tested by Fisher's exact test ($p < 0.0001$; Relative risk = 2.44; 95% CI: 1.80–3.30) (Fig. 1B). As to plasma HDL-C, the distribution analysis (low HDL <40 mg/dl versus high HDL ≥ 40 mg/dl) was not significantly deviated.

Correlation analysis of a population of 108 normolipidemic subjects from the same geographical area that we carried out preliminarily did not reach statistical significance (data not shown), suggesting that the ITIH4 may exert its modifying effect only in the hypercholesterolemic metabolic state. A subsequent, large-scale cohort study is obviously necessary to clarify whether or not the ITIH4 effect is also present in normolipidemic populations.

Discussion

Hypercholesterolemia is one of the important risk factors of ischemic heart disease (Matsuzawa 1995; Norioka 2000). In the present study, we showed a correlation between hypercholesterolemia and homozygosity for the C-allele of IVS17+8 C/T SNP in the *ITIH4* gene. An association study revealed an elevating effect of plasma T-Cho of the homozygosity for the C-allele. The high prevalence (93%) of hypercholesterolemia (mean±SD 252.3±23.5 mg/dl) among the individuals with this genotype (C/C) is in contrast to the scarceness (12%) of hypercholesterolemia (mean±SD 244.9±19.5 mg/dl and 200.4±39.7 mg/dl) among the rest of the study subjects (C/T and T/T). Our results indicated that *ITIH4* variation might modify the lipoprotein phenotype of plasma lipoprotein metabolism.

ITIH4 belongs to the inter-α-trypsin inhibitor family of serine protease inhibitors, which have diverse functions as an antiapoptotic and matrix-stabilizing molecule important throughout the development. Four genes, designated *ITIH1, 2, 3* and *4*, are involved in the synthesis of inter-α-trypsin inhibitor family members. All four heavy chain precursors and the resulting mature heavy chains harbor a so-called von Willebrand type-A (vWA) domain (Chan P et al. 1995, Bork P et al. 1991). Only the heavy chain 4 precursor polypeptide harbors a plasma Kallikrein-released bradykinin-like domain on its C-terminal sequence (Nishimura H et al. 1995) and a domain with some similarity to the ATP-dependent



	IVS17+8T-allele		Total
	(+)	(-)	
High T-Cho (percentage)	5 (12%)	286 (93%)	
Normal (percentage)	37 (88%)	23 (7%)	
Total (percentage)	42 (100%)	309 (100%)	351

B

($p < 0.0001$; Fisher's exact test)

Fig. 1A, B Comparison of plasma total cholesterol (T-Cho) levels among genotypically determined groups according to the *ITIH4* variation. A Adjusted plasma T-Cho levels were compared between genotypic groups with or without the ITIH4 IVS17+8 T-allele ($p=0.009$). Open circles represent mean values. Error bars represent the standard deviation (SD). B Distribution analysis of hypercholesterolemic patients among ITIH4 IVS17+8C/T classified-genotypes; T-allele (+)/(−). The 2×2 table was analyzed by Fisher's exact test ($p<0.0001$). High T-Cho phenotypes were defined by adjusted plasma T-Cho levels above the 220 mg/dl

proteases (Saguchi K et al. 1995). Although the suspected link between functional changes of ITIH4 protein and plasma T-Cho metabolism was not a commonly expected one, it may have unidentified functions in regulation of cholesterol synthesis and catabolism in the liver.

The IVS17+8C/T SNP locates close to the splice junction for the donor site of exon 17 of the *ITIH4* gene. Due to this polymorphic sequence near the splice junction, the *ITIH4* gene may have differential splicing that results in production of distinct ITIH4 isoforms in some organs. This possibility may need to be investigated by functional studies in the future. In addition, possibilities that this SNP marker may itself be in linkage disequilibrium with other unexamined functional variants localized in its close proximity or those in nearby genes have to be tested. HapMap initiative is now in progress among the international consortium. Haplotypic knowledge of the human genome will help clarify the contribution of the *ITIH4* gene in lipid profile through detailed haplotype association study in the near future. It would also be important to verify the association in geographically distinct populations and other ethnic groups in the future (Shastry 2002).

In summary, we noted an association between the variation of the inter-α-trypsin inhibitor heavy chain 4 (*ITIH4*) gene and hypercholesterolemia in 351 subjects from an area in the east-central region of Japan. Given our genetic results, we expect that the effects of multiple genes, both additive and interactive (Lalouel et al. 2001; Takada et al. 2002), will eventually prove to be responsible for many cases of common, inherited, mixed dyslipidemias.

Acknowledgements We thank Mitsuko Kajita, Mina Kodaira, Miho Kawagoe, Tamami Uchida, Kyoko Shimizu, Mayumi Tanaka, and Naoko Tsuruta for their expert technical assistance. This work was supported by a grant for Strategic Research from the Ministry of Education, Science, Sports and Culture of Japan; by a Research Grant for Research from the Ministry of Health and Welfare of Japan; by a Research for the Future Program Grant of The Japan Society for the Promotion of Science.

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