Genes Genom (2015) 37:173–181
DOI 10.1007/s13258-014-0234-5

RESEARCH ARTICLE

Effect of interactions between genetic polymorphisms and cigarette smoking on plasma triglyceride levels in elderly Koreans: the Hallym Aging Study

Eun Pyo Hong · Ji Wan Park · Jun-Gyo Suh · Dong-Hyun Kim

Received: 27 September 2014 / Accepted: 10 October 2014 / Published online: 28 October 2014

Abstract High triglyceride (TG) levels are known to be associated with complex diseases such as cardiovascular disease. Here, we evaluated the effect of interactions between genetic polymorphisms and environmental factors on plasma TG levels in a Korean aging cohort. Thirty-two single nucleotide polymorphism (SNP) markers located in seven genes (*ADLH2*, *APOE*, *GCKR*, *MC4R*, *TCF7L2*, *GATA2*, and *HNF1A*) for TG levels were genotyped in 714 older Koreans (mean age, 68 years). We performed multiple linear regression analyses for these SNP markers under three genetic models (i.e. additive, dominant, and recessive) and evaluated their associations with multiple environmental factors, including obesity, systolic blood pressure, and drinking and smoking habits. We found evidence for four SNP-smoking interactions. The CC genotype of rs1260326 (*GCKR*) interacted with current smoking for lower TG levels ($P_{G\times E} = 0.017$). TG levels were higher in smokers with the GG genotype of rs2713604 (*GATA2*) than in those with the GA + AA genotypes (195.6 and 145.7 mg/dL, respectively, $P_{G\times E} = 0.029$). The GA + AA genotypes of rs2713603 (*GATA2*) were associated with elevated TG levels in smokers when compared to non-smokers carrying the GG genotype (180.6 mg/dL vs. 148.3 mg/dL, $P_{G\times E} = 0.046$). The C allele of rs2464196 (*HNF1A*) interacted with current smoking for elevated TG levels ($P_{G\times E} = 0.028$). The gene and cigarette smoking interactions shown in this study need to be validated in a large-scale study.

Keywords Aging cohort · Cigarette smoking · Gene-environment interaction · Single nucleotide polymorphism · Triglycerides

E. P. Hong · J. W. Park · J.-G. Suh
Department of Medical Genetics, College of Medicine, Hallym University, Chuncheon, Gangwon-do 200-702, Republic of Korea

D.-H. Kim (✉)
Department of Social and Preventive Medicine, College of Medicine, Hallym University, Chuncheon, Gangwon-do 200-702, Republic of Korea
e-mail: dhkims@hallym.ac.kr

D.-H. Kim
Hallym Research Institute of Clinical Epidemiology, Hallym University, Chuncheon, Gangwon-do 200-702, Republic of Korea

Introduction

Global mortality due to chronic diseases, such as metabolic syndrome (MetS) and cardiovascular disease (CVD), is increasing in the twenty first century with the aging population (Partridge et al. 2011). An elevated plasma triglyceride (TG) level (above 150 mg/dL) is a well-known risk factor for CVD and MetS in the general population (Hokanson and Austin 1996; Grundy et al. 1999; Woodward et al. 2005). Willett et al. (1983) reported that cigarette smoking significantly increased TG levels in white women. Wu et al. (2001) found a strong association between increased TG levels and smoking and drinking habits in Chinese men. Previous Korean studies reported a positive association between TG levels and cigarette smoking (Park and Kang 2004; Oh et al. 2005). Park and Kang (2004) showed that elevated TG levels were significantly associated with DNA damage in current smokers.

TG levels are affected by multiple genetic and environmental factors (Iselius et al. 1985; Heller et al. 1993). Previous genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) in several

genes such as glucokinase (hexokinase 4) regulator (*GCKR*, 2p23), transcription factor 7-like 2 (*TCF7L2*, 10q25.3), and aldehyde dehydrogenase 2 family (*ALDH2*, 12q24.2) as well as the apolipoprotein cluster (*APOC*) and apolipoprotein E (*APOE*), which are both located in chromosomal region 19q13.2, that are associated with TG levels in Asian populations (Ng et al. 2008; Kathiresan et al. 2009; Teslovich et al. 2010). Two variants, rs12970134 and rs17782313, located near the melanocortin 4 receptor gene (*MC4R*, 18q22) were significantly associated with obesity-related traits such as TG level in Indians (Janipalli et al. 2012). Two recent studies replicated the association of several genetic variants (*APOB*, *GCKR*, *LPL*, *APOE*, etc.) with TG levels in Korean populations (Park et al. 2011; Kim et al. 2011).

Previous population-based studies have reported that several genetic variants interacted with cigarette smoking for increased TG levels. Waterworth et al. (2000) showed that alleles 3238G (rs5128) and -482T (rs2854117) in apolipoprotein C-III (*APOC3*, 11q23.1) interacted with smoking habit to increase TG levels in middle-aged men ($P = 0.042$ and $P = 0.009$, respectively). The common variant, H + H + genotype of the HindIII (rs320) SNP located in the lipoprotein lipase gene (*LPL*, 8p22) increased serum TG levels among smoking men in a Spanish cohort (Ariza et al. 2010). Tan et al. (2012) identified several genetic variants (*LPL*, *ALDH2*, *APOA5*, etc.) and environmental factors (i.e. smoking and drinking habits) for serum TG levels in a healthy Chinese male GWAS. For example, one SNP, the GG genotype of rs671 (*ALDH2*), was associated with increased serum TG levels in current drinkers ($P = 3.3 \times 10^{-5}$). Furthermore, none of the lipid-associated genetic variants, such as *PCSK9*, *APOB*, *TRIB1*, *FADS1*, *LDLR*, and *APOC1*, that have been identified in European populations, showed interactions with drinking or smoking for TG levels in Koreans (Park et al. 2011). However, the number of gene-environment interaction studies on TG levels in Koreans is limited, since most genetic variants were selected based on European populations.

In the current study, we evaluated the effects of genetic and environmental factors on plasma TG levels in 664 elderly Koreans (mostly over 60 years old). We also investigated gene and cigarette smoking interactions on plasma TG levels.

Materials and Methods

Study subjects and data collection

A population-based prospective cohort study, the Hallym Aging Study (HAS) recruited elderly individuals with a mean age of 68 years mostly living in Chuncheon city, Republic of Korea. Chuncheon city is divided into 1,408 areas based on the 2,000 population census, and 200 of these areas were randomly selected. The HAS was designed to investigate the quality of life and healthcare in this elderly population. The initial study population included 1,510 individuals (451 were aged 45 to 64 years, and 1,059 were over 65 years of age). A second panel survey was conducted in 2004, which included 918 adults (437 men and 534 women). A study flow diagram is shown in Fig. 1.

After informed consent was obtained from each participant, we collected self-reported questionnaires, anthropometric measurements, and clinical traits, including plasma TG levels, from each participant at three-year intervals. Questionnaires were administered by interviewers face-to-face. Anthropometric and clinical traits were measured by a clinical team at Chuncheon Sacred Heart Hospital. The study protocols and procedures were approved by the Institutional Review Board of Hallym University (HIRB-2007-001).

Clinical measurements

BMI was calculated as an individual's weight in kilograms divided by the square of his/her height in meters. We averaged three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) in one sitting using a standard protocol. We stored blood samples in a −70 °C deep freezer until analysis (DF8510; ilShinBioBase Co., Ltd., Korea). Plasma concentrations of total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and fasting blood sugar (FBS) were measured by an automatic analyser (Hitachi 7600-210; Hitachi Medical Corp., Hitachi, Japan) using enzymatic methods. Plasma insulin was estimated with Elecsys E170 (Hitachi Medical Corp.) and Versamax (Molecular Devices, Sunnyvale, CA, USA) systems. We estimated the homeostasis model assessment of insulin resistance index (HOMO-IR) using the formula described by Matthews et al. (1985). Other clinical data and procedures have been described elsewhere (Cho et al. 2009).

Genotyping and quality control

We extracted genomic DNA (gDNA) from peripheral blood or buffy coat using the FlexiGene DNA Extraction Kit (Qiagen Inc., Valencia, CA, USA), and DNA concentration was then estimated using a Nanobiometer (MECASYS Co., Ltd., Korea). The gDNA of each sample was separated by 1 % agarose gel electrophoresis to assess DNA quality. gDNA was quantified on a spectrofluorometer (Perkin Elmer Inc., CT, USA) by using the PicoGreen dsDNA quantification kit (Invitrogen Corp., Carlsbad, CA, USA). We excluded samples with low DNA concentrations (<50 ng/μL) before the genotyping test.

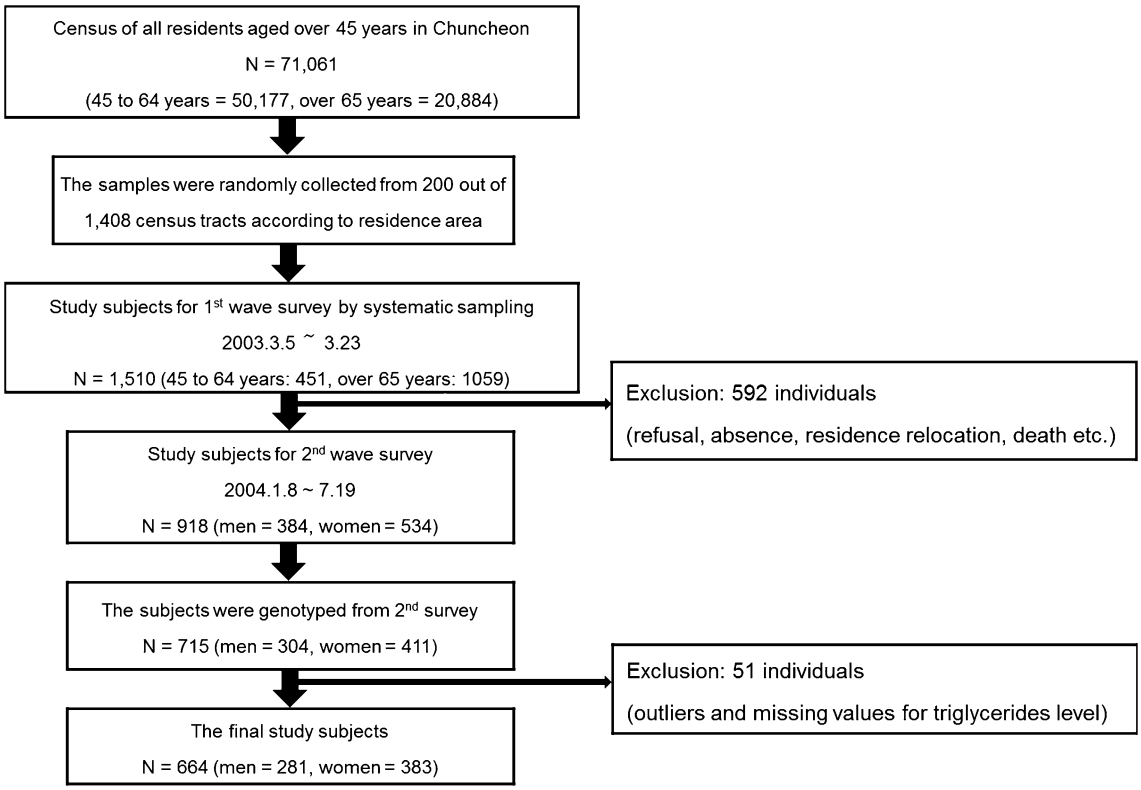

Fig. 1 Flow chart of the Hallym Aging Study

We selected three to ten SNPs located within 2 kb upstream and downstream of each of five candidate genes, *ADLH2*, *APOE*, *GCKR*, *MC4R*, and *TCF7L2* for TG levels, and two genes of interest (*GATA2* and *HNF1A*) for cigarette smoking by using the literature database, HuGE Navigator (http://hugenavigator.net/HuGENavigator/home.do) and dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/). We genotyped 715 Koreans for 32 SNPs with the Golden Gate Genotyping Assay (Illumina Inc., San Diego, CA, USA). We excluded three individuals and three SNPs with genotyping call rates <95 % (rs3751150, rs3751151, and rs4420638). We evaluated pair-wise linkage disequilibrium (LD), Hardy–Weinberg equilibrium (HWE), and minor allele frequency (MAF) by using the Haploview software package, v. 4.2 (Barrett et al. 2005).

Statistical analysis

We conducted univariate and multivariate analyses using the STATA software package, v. 11.2 (StataCorp., College Station, TX, USA). We excluded TG levels of outliers (mean + 3SD) and missing values in the baseline survey (N = 664). We transformed TG level to a natural logarithmic scale (logTG) to normalize the distribution of data. We performed a trend test for the median TG level according to smoking status habit using a one-sided Jonckheere-Terpstra test (Pirie 1983). To detect confounding factors, we performed single linear regression (SLR) analyses to evaluate associations of logTG with age, gender, history of hypertension, four lifestyle factors (regular exercise, smoking, drinking, and sleep), and eight clinical variables (BMI, waist circumference, SBP, DBP, TC, HDL-C, albumin, FBS, fasting insulin, and HOMA-IR). We identified a best-fit model using a general linear model (GLM) with stepwise backward elimination to estimate the beta coefficient (β) and 95 % confidence interval (CI) after adjusting for confounding factors.

We conducted a single SNP analysis to identify the associations of 27 SNP markers near or within seven genes with logTG levels under three genetic models (i.e. additive, dominant, and recessive) after adjustment for age and sex (GLM 1); and for the best-fitting GLM 2. To account for multiple testings, we used a significance threshold less than 0.0018 based on Bonferroni correction. We finally evaluated the interactions of 27 SNP markers with smoking habit on plasma TG levels in 664 individuals.

Results

The baseline characteristics of 664 subjects along with the TG levels are shown in Table 1. TG levels did not differ

Table 1 Baseline characteristics of study subjects in the Hallym Aging Study, 2007

Characteristic[a]	Male	Female	Total
Subject, N (%)	281 (42.3)	383 (57.7)	664 (100)
Age, years	69.0 ± 7.8	68.0 ± 9.6	68.4 ± 8.9
Hypertension, N (%)	85 (30.3)	121 (31.6)	206 (31.0)
Smoking status, N (%)			
Nonsmoker	53 (18.9)	346 (90.3)	399 (60.1)
Ex-smoker	144 (51.2)	17 (4.4)	161 (24.3)
Current smoker	84 (29.9)	20 (5.2)	104 (15.7)
Drinking status, N (%)			
Nondrinker	60 (21.3)	296 (77.3)	356 (53.6)
Ex-drinker	52 (18.5)	15 (3.9)	67 (10.1)
Current drinker	167 (59.4)	69 (18.0)	236 (35.5)
Sleeping status, N (%)			
>8 h	183 (65.1)	249 (65.0)	432 (65.1)
<8 h	89 (31.7)	119 (31.1)	208 (31.3)
Regular exercise, N (%)	67 (23.8)	47 (12.3)	114 (17.2)
Body mass index, kg/m^2	24.6 ± 2.9	25.3 ± 3.7	25.0 ± 3.4
Waist-hip ratio	0.93 ± 0.07	0.91 ± 0.08	0.92 ± 0.07
Systolic blood pressure, mmHg	137.0 ± 18.6	134.5 ± 19.7	135.6 ± 19.2
Diastolic blood pressure, mmHg	81.4 ± 11.4	80.2 ± 12.0	80.7 ± 11.8
Total cholesterol, mg/dL	192.2 ± 35.2	206.4 ± 36.3	200.4 ± 36.5
High density lipoprotein cholesterol, mg/dL	49.9 ± 18.5	51.6 ± 15.9	50.8 ± 17.0
Albumin, g/dL	4.59 ± 0.34	4.59 ± 0.28	4.59 ± 0.30
Fasting blood sugar, mg/dL	104.7 ± 28.1	101.1 ± 25.8	102.6 ± 26.8
Fasting blood insulin, μLU/mL	5.15 ± 15.03	5.12 ± 4.07	5.13 ± 10.29
HOMA-IR, mg/dL × μLU/mL[b]	1.40 ± 3.41	1.36 ± 1.35	1.38 ± 2.43
Triglycerides, mg/dL	166.2 ± 84.1	156.4 ± 73.3	160.5 ± 78.1

[a] The number of subjects (percentage) or mean ± standard deviation

[b] Homeostasis model assessment of insulin resistance

Table 2 Variation in the plasma triglyceride levels according to smoking status by gender group, Hallym Aging Study, 2007

Smoking status	Subjects, N	Triglyceride level, mg/dL[a]	P^{b}_{trend}
Men			
Nonsmoker	53	168.8 ± 82.1	
Ex-smoker	144	168.0 ± 84.8	
Current smoker	84	161.4 ± 84.1	0.758
Women			
Nonsmoker	346	155.0 ± 73.0	
Ex-smoker	17	158.4 ± 53.3	
Current smoker	20	179.5 ± 90.1	0.082
Total			
Nonsmoker	399	156.8 ± 74.3	
Ex-smoker	161	166.9 ± 82.0	
Current smoker	104	164.9 ± 85.7	0.112

[a] Mean and standard deviation for plasma triglyceride levels after excluding triglyceride level outlier

[b] *P* value for the trend test of triglyceride level by smoking habit using a one-sided Jonckheere-Terpstra test

between men and women (166.2 vs. 156.4 mg/dL, $P > 0.05$). Variations in the mean TG level according to smoking status in each group are shown in Table 2. Plasma TG levels did not differ by smoking status in any group ($P > 0.05$). Among 17 variables, we observed statistically significant associations for ten variables with *P* values less than 0.05 in SLR analyses (data not shown). The best-fitting model included six variables, age, sex, SBP, TC, HDL, and FBS, by using stepwise backward elimination ($6 \times 10^{-25} < P < 0.05$).

All of SNP markers that were near or within seven genes (i.e. *ADLH2*, *APOE*, *GCKR*, *MC4R*, *TCF7L2*, *GATA2*, and *HNF1A*) showed HWE *P* values greater than 0.05 and MAFs >1 %. 24 SNPs were described after considering LD (Table 3). We identified significant associations for seven SNPs with plasma TG levels of 664 individuals after adjustment for age and sex. Three variants, rs2713604 (*GATA2*), rs2713603 (*GATA2*), and rs3751152 (*HNF1A*), were not significant after adjusting for age, sex, SBP, TC, HDL, and FBS. For the four remaining SNPs, the CT and TT genotypes of rs7412 (*APOE*) showed the strongest

Table 3 Association of 24 SNPs with plasma triglyceride levels in 664 subjects

Gene Chr	SNP	Genetic model[a]	Function	M/m[b]	MM/Mm/mm[b]	HWE p[c]	MAF[c]	GLM 1[d]		GLM 2[d]	
								β	95 % CI	β	95 % CI
GCKR	rs2293571	D	Intron	C/T	498/155/11	1.00	0.13	0.04	−0.05, 0.13	0.04	−0.04, 0.11
2p23.3	rs1260326	A	Leu446Pro	T/C	204/336/124	0.53	0.44	0.02	−0.04, 0.07	0.02	−0.03, 0.07
	rs780092	D	Intron	T/C	308/303/53	0.08	0.31	0.05	−0.03, 0.12	0.04	−0.03, 0.11
GATA2	rs2713604	A	Intron	G/A	254/311/99	0.81	0.38	−0.06	−0.12, −0.01	−0.03	−0.08, 0.02
3q21.3	rs2713603	A	Intron	C/T	179/337/148	0.70	0.48	0.07	0.01, 0.12	0.03	−0.01, 0.08
	rs2860228	R	Intron	G/A	474/165/25	0.03	0.16	0.30	0.10, 0.49	0.23	0.05, 0.41
TCF7L2	rs10128255	D	Intron	A/G	374/252/38	0.68	0.25	0.03	−0.05, 0.10	0.01	−0.06, 0.07
10q25.3	rs7903146	A	Intron	C/T	634/29/1	0.30	0.02	−0.05	−0.22, 0.12	−0.07	−0.22, 0.08
	rs3750804	D	Intron	C/T	355/263/46	0.84	0.27	0.06	−0.02, 0.13	0.07	0.00, 0.13
	rs11594566	R	Intron	C/T	574/88/2	0.76	0.07	−0.43	−1.11, 0.25	−0.50	−1.10, 0.11
ALDH2	rs13306164	A	Leu105=	C/T	630/34/0	1.00	0.03	0.15	−0.01, 0.32	0.13	−0.02, 0.29
12q24.2	rs4648328	R	Intron	C/T	376/252/36	0.53	0.24	−0.01	−0.17, 0.16	0.05	−0.10, 0.20
	rs671	D (A)	Glu457Lys	G/A	456/190/18	0.89	0.17	−0.12	−0.20, −0.04	−0.12	−0.19, −0.04
	rs11066029	D	Intron	G/A	220/310/127	0.34	0.43	0.05	−0.03, 0.13	0.03	−0.05, 0.10
HNF1A	rs1169288	D	Ile27Leu	T/G	194/323/144	0.70	0.46	−0.02	−0.10, 0.06	−0.03	−0.10, 0.04
12q24.2	rs2393791[e]	R	Intron	A/G	198/337/129	0.53	0.45	0.11	0.01, 0.20	0.09	0.00, 0.17
	rs2464196[e]	D	Ser487Asn	T/C	161/352/150	0.12	0.49	−0.05	−0.13, 0.04	−0.03	−0.11, 0.05
	rs3751152	D	Asn227=	C/T	480/172/12	0.54	0.15	−0.10	−0.18, −0.02	−0.04	−0.12, 0.04
MC4R	rs8087522	R	Near Gene-5	G/A	448/201/15	0.22	0.17	−0.03	−0.28, 0.22	0.06	−0.17, 0.28
18q22	rs11872992	R	Near Gene-5	G/A	419/208/37	0.10	0.21	−0.11	−0.27, 0.05	−0.10	−0.24, 0.04
APOE	rs434132	A	Near Gene-5	G/C	644/19/0	1.00	0.01	0.01	−0.21, 0.24	−0.01	−0.21, 0.19
19q13.2	rs429358	R	Cys130Arg	T/C	548/113/1	0.05	0.09	0.52	−0.44, 1.47	0.51	−0.34, 1.36
	rs7412	D (A)	Arg176Cys	C/T	566/92/1	0.24	0.07	0.12	0.02, 0.23	0.21	0.11, 0.30
	rs439401	R	Near Gene-3	T/C	247/316/100	1.00	0.39	0.06	−0.04, 0.17	0.07	−0.02, 0.17

β beta coefficient, *Chr* chromosome, *CI* confidence interval, *GLM* general linear model, *HWP* Hardy–Weinberg equilibrium, *MAF* minor allele frequency

[a] The most significant genetic model for generalized linear model 2 (GLM 2) is shown, and any other significant model is shown in parentheses: *A* additive, *D* dominant, *R* recessive

[b] M/m denotes the major/minor allele type. MM/Mm/mm denotes number of individuals with the common homozygote/heterozygote/rare homozygote genotypes

[c] HWPp, Hardy–Weinberg equilibrium *P* value; MAF, minor allele frequency

[d] Beta coefficients and 95 % confidence intervals estimated from generalized linear regression analyses after adjustment for age and sex (GLM 1); for age, sex, SBP, TC, HDL, and FBS (GLM 2)

[e] Only one SNP is shown when multiple SNPs were in strong linkage disequilibrium (LD): rs2393791 and rs7310409 ($r^2 = 0.99$); rs2464196 with rs2259816 and rs2259820 ($r^2 = 0.98$ and 0.99, respectively)

association with increased TG levels under the dominant model (β = 0.21; 95 % CI, 0.11–0.30; $P = 1.9 \times 10^{-5}$). In addition, we found that two novel genetic variants, the AA genotype of rs2860288 (*GATA2*) and the GG genotype of rs2393791 (*HNF1A*), increased plasma TG levels (β = 0.23; 95 % CI, 0.05–0.41 and β = 0.09; 95 % CI, 0.00–0.17, respectively). Two additional SNPs, rs671 (*ALDH2*) and rs7412 (*APOE*), remained significant after Bonferroni correction in GLM 2 ($P < 0.0018$). These variants lead to non-synonymous substitutions that modify the amino acid composition of the gene products (Table 3).

Among the 24 SNPs, we identified statistically significant interactions of four genetic variants, *GCKR* (rs1260326), *GATA2* (rs2713604 and rs2713603), and *HNF1A* (rs2464196) with smoking on plasma TG levels (Fig. 2 and Table 4). As shown in Table 4, while the mean TG levels of non-smokers who were C allele carriers of rs1260326 (*GCKR*) (G+) and of current smokers not carrying a C allele (E+) were 161.9 mg/dL and 172.7 mg/dL, respectively, smokers carrying the CC genotype (G+ E+) had significantly lower TG levels (124.6 mg/dL) (β = -0.33, $P_{G\times E} = 0.017$). Plasma TG levels were highest in current smokers with the CC genotype of rs2713604 (*GATA2*) (195.6 mg/L). The ancestral G and C alleles of rs2713603 and rs2464196 were associated with increased plasma TG levels in smokers (180 mg/dL, $P_{G\times E} = 0.046$

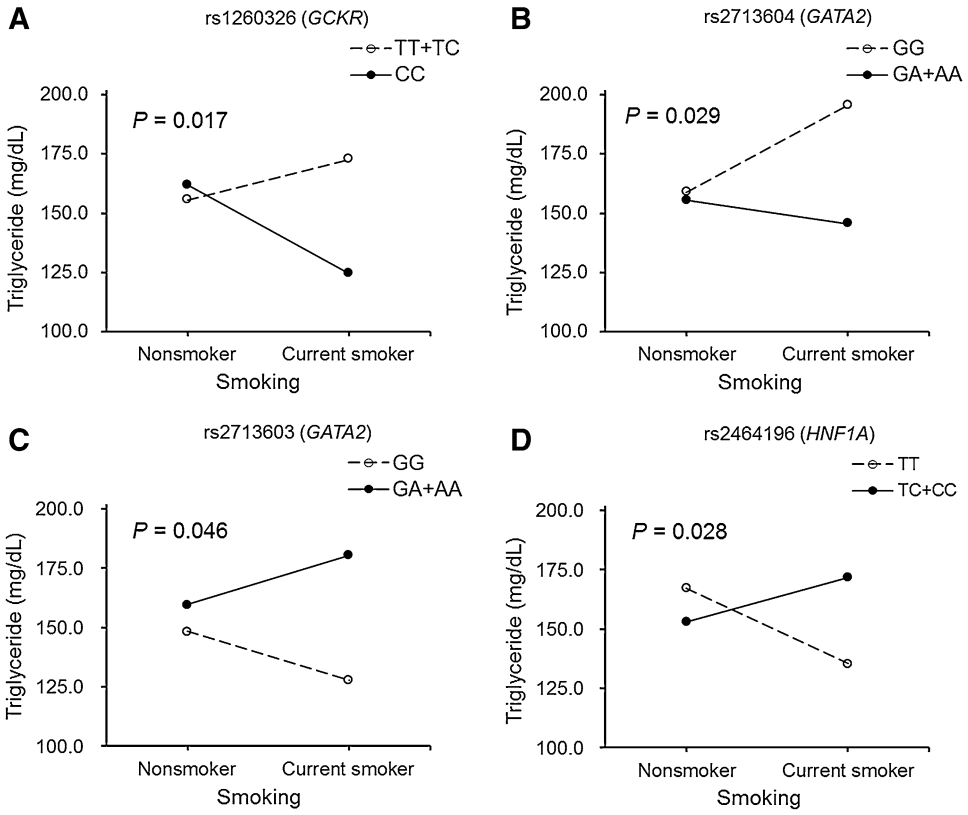

Fig. 2 The effect of interactions between four SNPs and smoking on triglyceride levels in Koreans. The interaction models shown in A–D are for rs1260326 (*GCKR*), rs2713604 (*GATA2*), rs2713603 (*GATA2*), and rs2464196 (*HNF1A*) with smoking status, respectively (*P*, *P* value for the interaction)

Table 4 Interactions between genetic polymorphisms and smoking status for plasma triglyceride levels

Gene	SNP	Genotype	Smoking	Subject, N	Triglycerides, mg/dL[a]	GLM 1[b]		
Chr			Status			β[b]	95 % CI[b]	*P* value[b]
GCKR	rs1260326	TT + TC	Nonsmoker	322	155.6		Reference	0.017
2p23.3			Current smoker	87	172.7	0.1	−0.02, 0.21	
		CC	Nonsmoker	77	161.9	0.06	−0.05, 0.18	
			Current smoker	17	124.6	−0.33	−0.60, −0.06	
GATA2	rs2713604	GG	Nonsmoker	158	158.9		Reference	0.029
3q21.3			Current smoker	40	195.6	0.18	0.02, 0.35	
		GA + AA	Nonsmoker	241	155.4	−0.01	−0.11, 0.08	
			Current smoker	64	145.7	−0.23	−0.44, −0.02	
	rs2713603	GG	Nonsmoker	103	148.3		Reference	0.046
			Current smoker	31	127.8	−0.12	−0.31, 0.07	
		GA + AA	Nonsmoker	296	159.8	0.06	−0.04, 0.17	
			Current smoker	73	180.6	0.23	0.00, 0.45	
HNF1A	rs2464196[c]	TT	Nonsmoker	107	167.3		Reference	0.028
12q24.2			Current smoker	20	135.5	−0.18	−0.41, 0.04	
		TC + CC	Nonsmoker	291	153.1	−0.11	−0.21, 0.00	
			Current smoker	84	171.8	0.28	0.03, 0.53	

[a] Mean plasma triglyceride levels

[b] Beta coefficients (βs) 95 % confidence intervals (CIs) and *P* values estimated from interaction analyses after adjustment for age and sex

[c] Only one SNP is shown when multiple SNPs were in strong linkage disequilibrium (LD): rs2464196 with rs2259816 and rs2259820 ($r^2 = 0.98$ and 0.99, respectively)

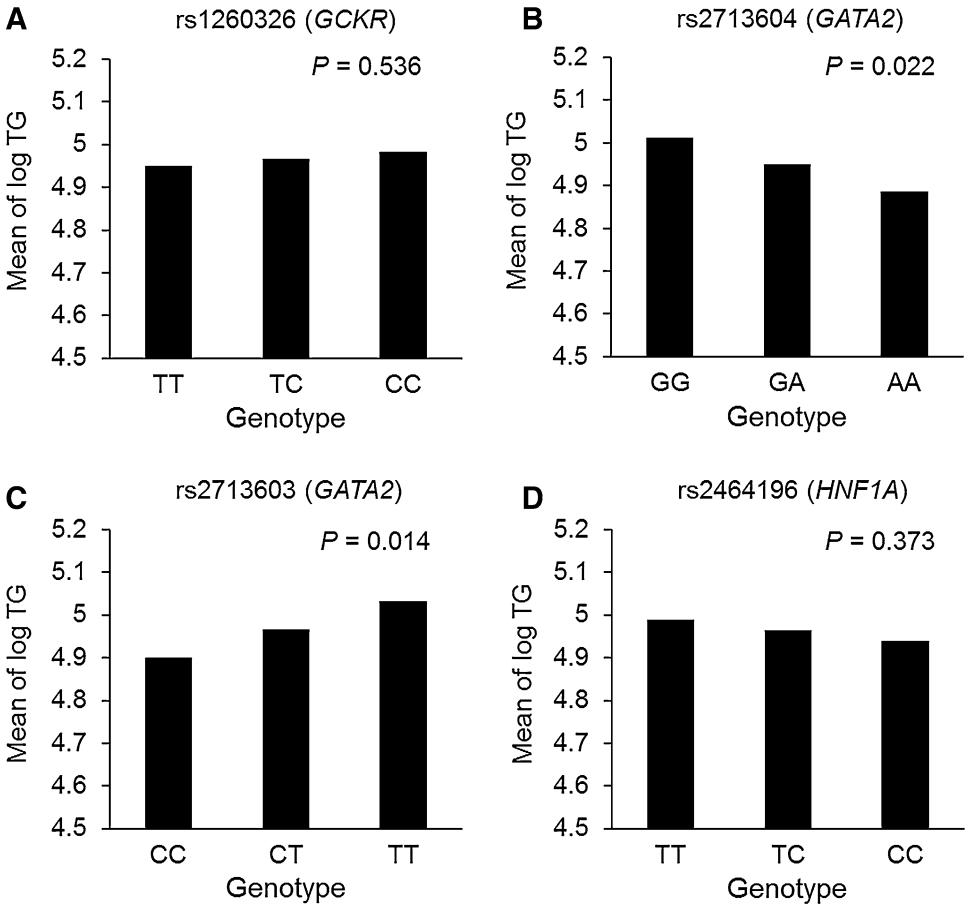

Fig. 3 Variations in the mean log-transformed triglyceride (logTG) levels according to each genotype. The mean logTG levels shown in A–D are for rs1260326 (*GCKR*), rs2713604 (*GATA2*), rs2713603 (*GATA2*), rs2464196 (*HNF1A*) after adjustment for age and sex, respectively (*P*, adjusted *P* value)

and 171 mg/dL, $P_{G\times E} = 0.028$, respectively). However, two missense variants in rs1260326 (Leu446Pro) and rs2464196 (Ser487Asn) showed no significant association with mean logTG after adjustment for age and sex ($P = 0.536$ and $P = 0.373$, respectively; Fig. 3).

Discussion

High TG levels can be caused by multiple risk factors such as obesity, family history, a lack of exercise, blood pressure, smoking, and genetic variants (Iselius et al. 1985; Heller et al. 1993). TG levels significantly predicted the risk of CVD and type 2 diabetes mellitus (T2DM) (Woodward et al. 2005; Pearson et al. 2003; Tirosh et al. 2008). Many prospective cohort studies have reported a positive association between elevated TG levels and cigarette smoking (Willett et al. 1983; Grundy et al. 1999; Wu et al. 2001; Oh et al. 2005). In the current study, plasma TG levels were not associated with smoking in 664 elderly Koreans.

This study showed six covariates that increased plasma TG levels in the best-fitting GLM (i.e. age, sex, SBP, TC, HDL, and FBS). We confirmed the associations between two gene variants (*ALDH2* and *APOE*) and plasma TG levels after adjustment for the GLM in this population (Teslovich et al. 2010; Tan et al. 2012), while the *GCKR*, *TCF7L2*, and *MC4R* polymorphisms were not significantly associated with TG levels. A missense variant, the AA genotype of rs671 (Glu504Lys) located in the *ALDH2* gene, was reported to be related to alcohol metabolism and associated with low TG levels in Chinese populations (Tan et al. 2012; Wang et al. 2013). The T allele of rs7412 (Arg176Cys, in *APOE*) has been reported to be associated with increased TG levels after adjustment for age, sex, waist circumference, blood glucose, blood pressure, and smoking and drinking status in an additive effect model as well as the risk of Alzheimer's disease (Ariza et al. 2010; Lescai et al. 2011). Interestingly, two novel genetic variants, the rare G and A alleles of rs2860288 (*GATA2*) and rs2393791 (*HNF1A*), respectively, showed a positive association with plasma TG levels in the recessive model. The *GATA2* gene (3q21.3) encodes a transcription factor, which contains six exons and plays a role in haematopoiesis and endothelial dysfunction (Connelly et al. 2006). Mutations in *GATA2* polymorphisms are associated with a

risk of familial early-onset coronary artery disease, sporadic monocytopenia, mycobacterial infection syndrome, and lung cancer (Connelly et al. 2006; Hsu et al. 2011; Kumar et al. 2012). In addition, this gene polymorphism was found to be strongly associated with smoking habit in a healthy US population (Pan et al. 2010). The *HNF1A* gene was shown to play a critical role in pancreatic β-cell function (Ryffel 2001) and is strongly associated with a risk of T2DM in smokers (Ley et al. 2011). Cauchi et al. (2008) showed obesity-modified associations between genetic variants (e.g. *GCKR* and *HFN1A*) and T2DM in European populations. Particularly, they demonstrated the impact of rs1169288 variants (*HNF1A*) on T2DM susceptibility in non-obese subjects.

Previous population-based studies showed that TG levels were modified by the interactions of several genetic variants (e.g. *LPL* and the *APOE-APOC* clusters) with cigarette smoking in general populations (Waterworth et al. 2000; Ariza et al. 2010). However, none of the genetic variants showed statistically significant interactions with cigarette smoking on serum TG level in Asian populations (Tan et al. 2012; Park et al. 2011). In this study, we observed novel interactions of rs1260326 (*GCKR*), rs2713604 (*GATA2*), rs2713603 (*GATA2*), and rs2464196 (*HNF1A*) with current smoking for plasma TG levels ($P < 0.05$). A limitation of this study is that the limited number of study subjects could increase the possibility of false negative results in the identification of gene-environment interactions. In addition, we were not able to determine the specific biological mechanisms underlying the statistically significant interactions between the genes and cigarette smoking on increased plasma TG levels.

In conclusion, common susceptibility variants and their interaction with smoking habits affect the inter-individual variability of plasma TG levels. The novel interactions between genetic variants (i.e. rs1260326:*GCKR*, rs2713604:*GATA2*, rs2713603:*GATA2*, and rs2464196:*HNF1A*) and cigarette smoking reported in the current study need to be validated in large-scale cohort studies. Particularly, verification of the interactions of some genetic variants with cigarette smoking on increased TG levels may be necessary to demonstrate the functional role of those variants in complex human diseases. Future studies focusing on the complex interactions between multiple genetic and environmental factors on TG levels could contribute to personalized preventive and therapeutic approaches to reduce CVD and MetS.

Acknowledgments This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology, Republic of Korea (2009-0090837) and the Hallym University Research Fund 2012 (HRF-G2012-5).

Conflict of interest None declared.

References

Ariza MJ, Sánchez-Chaparro MA, Barón FJ, Hornos AM, Calvo-Bonacho E, Rioja J, Valdivielso P, Gelpi JA, González-Santos P (2010) Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study. BMC Med Genet 11:66

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Cauchi S, Nead KT, Choquet H, Horber F, Potoczna N, Balkau B, Marre M, Charpentier G, Froguel P, Meyre D (2008) The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies. BMC Med Genet 9:45

Cho YG, Song HJ, Lee SK, Jang SN, Jeong JY, Choi YH, Hong KS, Choi MG, Kang SH, Kang JH et al (2009) The relationship between body fat mass and erectile dysfunction in Korean men: Hallym Aging Study. Int J Impot Res 21:179–186

Connelly JJ, Wang T, Cox JE, Haynes C, Wang L, Shah SH, Crosslin DR, Hale AB, Nelson S, Crossman DC et al (2006) GATA2 is associated with familial early-onset coronary artery disease. PLoS Genet 2:e139

Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, Mitch W, Smith SC Jr, Sowers JR (1999) Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 100:1134–1146

Heller DA, de Faire U, Pedersen NL, Dahlén G, McClearn GE (1993) Genetic and environmental influences on serum lipid levels in twins. N Engl J Med 328:1150–1156

Hokanson JE, Austin MA (1996) Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 3:213–219

Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, Frucht DM, Vinh DC, Auth RD, Freeman AF et al (2011) Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood 118:2653–2655

Iselius L, Carlson LA, Morton NE, Efendić S, Lindsten J, Luft R (1985) Genetic and environmental determinants for lipoprotein concentrations in blood. Acta Med Scand 217:161–170

Janipalli CS, Kumar MV, Vinay DG, Sandeep MN, Bhaskar S, Kulkarni SR, Aruna M, Joglekar CV, Priyadharshini S, Maheshwari N et al (2012) Analysis of 32 common susceptibility genetic variants and their combined effect in predicting risk of Type 2 diabetes and related traits in Indians. Diabet Med 29:121–127

Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T et al (2009) Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 41:56–65

Kim YJ, Go MJ, Hu C, Hong CB, Kim YK, Lee JY, Hwang JY, Oh JH, Kim DJ, Kim NH et al (2011) Large-scale genome-wide association studies in East Asians identify new genetic loci influencing metabolic traits. Nat Genet 43:990–995

Kumar MS, Hancock DC, Molina-Arcas M, Steckel M, East P, Diefenbacher M, Armenteros-Monterroso E, Lassailly F, Matthews N et al (2012) The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small cell lung cancer. Cell 149:642–655

Lescai F, Chiamenti AM, Codemo A, Pirazzini C, D'Agostino G, Ruaro C, Ghidoni R, Benussi L, Galimberti D, Esposito F et al (2011) An APOE haplotype associated with decreased ε4

expression increases the risk of late onset Alzheimer's disease. J Alzheimers Dis 24:235–245

Ley SH, Hegele RA, Harris SB, Mamakeesick M, Cao H, Connelly PW, Gittelsohn J, Retnakaran R, Zinman B, Hanley AJ (2011) HNF1A G319S variant, active cigarette smoking and incident type 2 diabetes in Aboriginal Canadians: a population-based epidemiological study. BMC Med Genet 12:1

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419

Ng MC, Park KS, Oh B, Tam CH, Cho YM, Shin HD, Lam VK, Ma RC, So WY, Cho YS et al (2008) Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, and FTO in type 2 diabetes and obesity in 6,719 Asians. Diabetes 57:2226–2233

Oh SW, Yoon YS, Lee ES, Kim WK, Park C, Lee S, Jeong EK, Yoo T (2005) Association between cigarette smoking and metabolic syndrome: the Korea National Health and Nutrition Examination Survey. Diabetes Care 28:2064–2066

Pan F, Yang TL, Chen XD, Chen Y, Gao G, Liu YZ, Pei YF, Sha BY, Jiang Y, Xu C et al (2010) Impact of female cigarette smoking on circulating B cells in vivo: the suppressed ICOSLG, TCF3, and VCAM1 gene functional network may inhibit normal cell function. Immunogenetics 62:237–251

Park E, Kang MH (2004) Smoking and high plasma triglyceride levels as risk factors for oxidative DNA damage in the Korean population. Ann Nutr Metab 48:36–42

Park MH, Kim N, Lee JY, Park HY (2011) Genetic loci associated with lipid concentrations and cardiovascular risk factors in the Korean population. J Med Genet 48:10–15

Partridge EE, Mayer-Davis EJ, Sacco RL, Balch AJ (2011) Creating a twenty first century global health agenda: the general assembly of the united nations high level meeting on non-communicable diseases. Circulation 123:3012–3014

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL et al (2003) Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. Circulation 107:499–511

Pirie W (1983) Jonckheere tests for ordered alternatives. In: Kotz S, Johnson NL (eds) Encyclopedia of statistical sciences. John Wiley and Sons, New York, pp 315–318

Ryffel GU (2001) Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. J Mol Endocrinol 27:11–29

Tan A, Sun J, Xia N, Qin X, Hu Y, Zhang S, Tao S, Gao Y, Yang X, Zhang H et al (2012) A genome-wide association and gene-environment interaction study for serum triglycerides levels in a healthy Chinese male population. Hum Mol Genet 21:1658–1664

Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ (2010) Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466:707–713

Tirosh A, Shai I, Bitzur R, Kochba I, Tekes-Manova D, Israeli E, Shochat T, Rudich A (2008) Changes in triglyceride levels over time and risk of type 2 diabetes in young men. Diabetes Care 31:2032–2037

Wang Y, Zhang Y, Zhang J, Tang X, Qian Y, Gao P, Zhu D (2013) Association of a functional single-nucleotide polymorphism in the ALDH2 gene with essential hypertension depends on drinking behavior in a Chinese Han population. J Hum Hypertens 27:181–186

Waterworth DM, Talmud PJ, Bujac SR, Fisher RM, Miller GJ, Humphries SE (2000) Contribution of apolipoprotein C-III gene variants to determination of triglyceride levels and interaction with smoking in middle-aged men. Arterioscler Thromb Vasc Biol 20:2663–2669

Willett W, Hennekens CH, Castelli W, Rosner B, Evans D, Taylor J, Kass EH (1983) Effects of cigarette smoking on fasting triglyceride, total cholesterol, and HDL-cholesterol in women. Am Heart J 105:417–421

Woodward M, Huxley H, Lam TH, Barzi F, Lawes CM, Ueshima H (2005) A comparison of the associations between risk factors and cardiovascular disease in Asia and Australasia. Eur J Cardiovasc Prev Rehabil 12:484–491

Wu DM, Pai L, Sun PK, Hsu LL, Sun CA (2001) Joint effects of alcohol consumption and cigarette smoking on atherogenic lipid and lipoprotein profiles: results from a study of Chinese male population in Taiwan. Eur J Epidemiol 17:629–635