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Assessment of Relationship between Fyn-related Kinase Gene Polymorphisms and Overweight/Obesity in Korean Population

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The fyn-related kinase (FRK) belongs to the tyrosine kinase family of protein kinases. Recent studies have shown that Frk affects pancreatic beta cell number during embryogenesis and promotes beta cell cytotoxic signals in response to streptozotocin. To investigate the genetic association between *FRK* polymorphisms and the risk of obesity in Korean population, single nucleotide polymorphisms (SNPs) in the *FRK* gene region were selected and analyzed. The body mass index (BMI) was calculated, and biochemical data (systolic blood pressure, diastolic blood pressure, hemoglobin A1C, triglyceride, total cholesterol, high density lipoprotein, and low density lipoprotein) of blood sample from each subject were also measured. One hundred fifty five healthy control and 204 overweight/obesity subjects were recruited. Genotype frequencies of six SNPs [rs6568920 (+8391G>A), rs3756772 (+56780A>G), rs3798234 (+75687C>T), rs9384970 (+68506G>A), rs1933739 (+72978G>A), and rs9400883 (+75809A>G)] in the *FRK* gene were determined by Affymetrix Targeted Genotyping Chip data. According to the classification of Korean Society for the Study of Obesity, control (BMI 18 to <23) and overweight/obesity (BMI≥23) subjects were recruited. For the analysis of genetic data, EM algorithm, SNPStats, Haploview, HapAnalyzer, SNPAnalyzer, and Helixtree programs were used. Multiple logistic regression analysis (codominant, dominant, and recessive models) was performed. Age and gender as covariates were adjusted. For biochemical data, Student's *t* test was used. The mean value of BMI in the control and overweigh/obesity groups was 21.1±1.2 (mean±SD) and 25.6±2.0, respectively. All biochemical data of the overweight/obesity group were statistically significance, compared with the control group. Among six SNPs, two linkage disequilibrium (LD) blocks were discovered. One block consisted of rs1933739 and rs9400883, and the other comprised rs3756772 and rs3798234. One SNP (rs9384970, +68506G>A) showed an association with overweight/obesity in the codominant model (p=0.03). Interestingly, the AA genotype distribution in the overweight/obesity group (n=7, 3.5%) was higher than those in the control group (n=1, 0.6%), which is not found in either Japanese or Chinese subjects. Therefore, the AA genotype of rs9384970 may be a risk factor for development of obesity in Korean population. The results suggest that *FRK* may be associated with overweight/obesity in Korean population.

Key Words: Body mass index, Fyn-related kinase, Linkage disequilibrium, Obesity, Overweight, Single nucleotide polymorphism

INTRODUCTION

Obesity is a common and rapidly growing health problem, which raises the mortality due to diabetes, hypertension, and cardiovascular diseases (Burton et al, 1985; Alberti & Zimmet, 1998). Obesity is exacerbated by physical inactivity, advancing age, and endocrine dysfunction. The tendency for weight gain is more likely due to influences of multiple genes and environmental factors (Hubert et al, 1983; Flegal et al, 1998). Nevertheless, the relationship between obesity and diabetes has well been established: obesity is a major risk factor for the development of diabetes, and approximately 80% of type 2 diabetes is overweight or obese (Flegal et al, 1998; Mokdad et al, 1999). Insufficient insulin causes deficiencies in pancreatic beta cell function or insulin secretion (Bell et al, 2001; Kahn, 2003).

The fyn-related kinase (*FRK*; other aliases, *GTK, PTK5, RAK*) is located on chromosome 6q21-q22.3. The Frk is a tyrosine kinase expressed in various tissues, such as the gastrointestinal tract and pancreatic islet cells (Thuveson et al, 1995). The Frk has been shown to induce beta cell cytotoxic signals in response to several cytokines and beta cell toxin, streptozotocin (Annerén, 2002; Annerén & Welsh,

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ABBREVIATIONS: BMI, body mass index; CI, confidence interval; LD, linkage disequilibrium; FRK, fyn-related kinase; OR, odds ratio; SNP, single nucleotide polymorphism.

2002). Annerén et al (2007) reported that transgenic mice expressing the Frk tyrosine kinase under the control of the rat insulin promoter showed glucose intolerance and reduced islet blood flow, and Akerblom et al (2007) demonstrated with Frk knockout (−/−) mice that Frk regulates beta cell number during embryogenesis in early life. Recently, polymorphisms of *FRK* have been shown to be related with the development of schizophrenia or Alzheimer's disease (Nakano et al, 2004; Rybakowski et al, 2007). However, no study has yet been carried out on the possible relationship between *FRK* gene and obesity. In this study, therefore, an association between single nucleotide polymorphisms (SNPs) of the *FRK* gene and overweight /obesity in Korean population was investigated.

METHODS

Subjects

The body mass index (BMI) of each subject was calculated from height and weight using the formula: BMI=kg/m^2. As per the Classification of Korean Society for the Study of Obesity (underweight, BMI <18; normal, BMI 18 to <23; moderately obese, BMI 23 to <25; obesity I, BMI 25 to <30; obesity II, BMI ≥30), normal control (BMI 18 to <23, n=155) and overweight/obesity (BMI ≥23, n=204) subjects were recruited at Kyung Hee University Medical Center. Patients with hypertension, diabetes, hyperlipidemia, stoke, and cardiac diseases were excluded. All studies were carried out according to the Declaration of Helsinki guidelines. All the subjects gave written informed consent before joining the study. This study was approved by the ethics committee of the Medical Research Institute, Kyung Hee University Medical Center.

Table 1. Clinical and biochemical characteristics of overweight/ obesity and control subjects

	Control (n=150)	Overweight/obesity (n=204)	p
Age (year)	43.7±6.2	44.8±6.4	0.087
BMI (kg/m^2)	21.1±1.2	25.6±2.0	<0.001
SBP (mmHg)	115.4±16.1	124.1±17.6	<0.001
DBP (mmHg)	71.9±10.3	77.8±11.3	<0.001
Fasting plasma glucose (mg/dl)	89.8±11.5	93.8±14.7	0.006
HbA1C (%)	5.3±0.4	5.5±0.7	0.028
TG (mg/dl)	97.5±56.7	140.3±117.2	<0.001
TC (mg/dl)	186.4±30.0	197.2±34.3	0.002
HDL (mg/dl)	56.9±13.1	49.8±11.3	<0.001
LDL (mg/dl)	109.4±28.8	119.3±32.5	0.002

Data are mean±SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1C, hemoglobin A1C; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

Biochemical measurements and DNA isolation

Blood samples were drawn for biochemical measurements: systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose, hemoglobin A1C (HbA1C), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), and high density lipoprotein (HDL). DNA samples were isolated by using Core One^{TM} Blood Genomic DNA Isolation Kit (CoreBioSystem^{TM}, Seoul, Korea).

SNP selection and genotyping

We initially selected 6 SNPs (rs6568920, rs3756772, rs3798234, rs9384970, rs1933739, and rs9400883) within the *FRK* gene region using the following websites: (1) human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP) (2) HapMap database (http://www.hapmap.org) (3) tag SNPs site (http:// broad.mit.edu/mpg/tagger). The SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded. The genotyping was performed using the Affymetrix Targeted Genotyping Chip array (Affymetrix, CA, USA). In brief, DNA was digested and then subjected to PCR using primers that were specific to the adaptor sequence. PCR products were purified, and the fragmented DNA was end-labeled with biotin using terminal deoxynucleotidyl transferase. Labeled DNA was then hybridized onto the Mapping Array. The hybridized array was washed, stained, and scanned

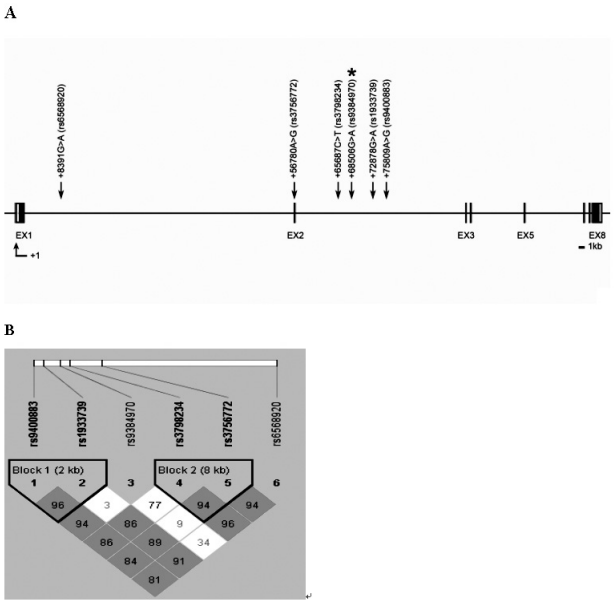

Fig. 1. Gene map and linkage disequilibrium (LD) in fyn-related kinase (*FRK*) gene. (A) Gene map of single nucleotide polymorphisms (SNPs) in *FRK* on chromosome 6q21-q22.3. Exons are marked with box. The coding regions are represented by black boxes and untranslation regions by white boxes. The first nucleotide of the transcriptional start site is denoted as +1. Asterisk (*) indicates a significant SNP. Arrow indicates the location of each SNP. EX, exon. (B) LD coefficient (| D' |) and LD blocks among *FRK* SNPs. Block 1 consists of rs9400883 and rs1933739. Block 2 comprises rs3798234 and rs3756772.

according to the manufacturer's instructions. The image was analyzed using GCOS software (Affymetrix).

Statistical analysis

Hardy-Weinberg equilibrium was calculated by SNPStats (http://bioinfo.iconcologia.net/index.php) (Solé et al, 2006). For the linkage disequilibrium (LD), Haploview version 3.32 was used (Barrett et al, 2005). The haplotypes and their frequencies were estimated using the EM algorithm (Stephens et al., 2001). To evaluate odds ratio (OR), 95% confidence intervals (CI), and p value, SNPStats, Hap-Analyzer version 1.0 (Jung et al, 2004), SNPAnalyzer (ISTECH Inc., Goyang, Korea), and Helixtree (Golden Helix Inc., MT, USA) were used. Clinical characteristics were compared between control and overweigh/obesity subjects, using Student's *t* test. For all statistical tests, the significant level was set at 0.05. Power analysis was performed using G*Power computer software (Faul et al, 2007).

RESULTS

Clinical and biochemical characteristics of control and overweight/obesity subjects are shown in Table 1. The mean value of BMI in the overweight/obesity group (25.6±2.0, mean±SD) was significantly higher than that in the control group (21.1±1.2). The levels of SBP, DBP, fasting plasma glucose, HbA1C, TG, TC, HDL, and LDL in overweight/obesity subjects were significantly different, compared to those in control subjects ($p<0.05$) (Table 1).

To assess whether polymorphisms of *FRK* have any correlation with the risk of obesity, genotype frequencies of 6 SNPs in the *FRK* gene were examined in 204 overweight/obesity and 155 control subjects, and multiple logistic regression analysis with adjustment for age and gender was performed. Six SNPs [rs6568920 (+8391G>A), rs3756772 (+56780A>G), rs3798234 (+75687C>T), rs9384970 (+68506G>A), rs1933739 (+72978G>A), and rs9400883 (+75809A>G)] of the *FRK* gene region are illustrated in Fig. 1A. The first nucleotide of the transcriptional initiation site of *FRK* is denoted as +1, and genotype distributions of all polymorphisms in this study were in Hardy-Weinberg equilibrium ($p>0.05$). As shown in Fig. 1B, two LD blocks were discovered by the Gabriel method (Gabriel et al, 2002). Haplotype analysis of block 1 (rs1933739 and rs9400883) and block 2 (rs3756772 and rs3798324) showed no significant association in any models

Table 2. Logistic regression analysis and genotype frequency of fyn-related kinase (*FRK*) polymorphisms in overweight/obesity and control subjects

Locus (SNP)	Region	Genotype	Overweight/ obesity n=204 (%)	Control n=150 (%)	Codominant		Dominant		Recessive	
					OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
+8391G>A	Intron1	GG	122 (60.4)	92 (61.3)	0.96	0.98	0.96	0.86	0.93	0.92
(rs6568920)		GA			(0.61~1.53)		(0.62~1.48)		(0.41~2.22)	
		AA	66 (32.7)	48 (32.0)						
			14 (6.9)	10 (6.7)						
+56780A>G	Exon2	AA	115 (56.4)	82 (54.7)	1.12	0.80	1.07	0.75	0.85	0.66
(rs3756772)		AG			(0.71~1.76)		(0.70~1.64)		(0.40~1.80)	
		GG	70 (34.3)	56 (37.3)						
			19 (9.3)	12 (8.0)						
+65687C>T	Intron2	CC	133 (65.5)	99 (66.0)	1.04	0.77	0.98	0.92	0.72	0.48
(rs3798234)		CT			(0.65~1.66)		(0.63~1.53)		(0.28~1.84)	
		TT	57 (28.1)	44 (29.3)						
			13 (6.4)	7 (4.7)						
+68506G>A	Intron2	GG	154 (76.2)	104 (69.3)	**1.63**	**0.03***	1.42	0.15	0.19	0.06
(rs9384970)		GA			**(0.99~2.66)**		(0.88~2.28)		(0.02~1.54)	
		AA	41 (20.3)	45 (30.0)						
			7 (3.5)	1 (0.7)						
+72878G>A	Intron2	GG	128 (64.7)	88 (61.1)	1.17	0.80	1.16	0.50	1.07	0.89
(rs1933739)		GA			(0.73~1.86)		(0.75~1.81)		(0.39~2.95)	
		AA	61 (30.8)	49 (34.0)						
			9 (4.5)	7 (4.9)						
+75809A>G	Intron2	AA	83 (42.9)	64 (44.4)	1.01	0.94	0.94	0.77	0.74	0.35
(rs9400883)		AG			(0.63~1.61)		(0.61~1.45)		(0.39~1.40)	
		GG	79 (41.8)	63 (43.8)						
			29 (15.3)	17 (11.8)						

Genotype distributions are shown as number (%). Odds ratio (OR), 95% confidence interval (CI), and p values were from logistic regression analysis with codominant, dominant, and recessive models controlling age and gender as covariates. Total number of each SNP is different, because genotypes of some SNPs are unreadable. SNP, single nucleotide polymorphism.

(data not shown). The genotype distributions of six SNPs in overweight/obesity and control subjects are shown in Table 2. Among six SNPs, one SNP (rs9384970, +68506G>A) presented an association with overweight/obesity in the codominant model (p=0.03, OR=0.21, 95% CI=0.02~1.70). The rest of the SNPs (rs6568920, rs3756772, rs3798234, rs1933739, and rs9400883) were not statistically associated with overweight/obesity (Table 2). We calculated the sample power for the significant SNP (rs9384970). In our case-control study, we had 0.844 (effect size w=0.174), assuming an α-level of 0.05. Thus, the rs9384970 SNP was sufficiently powerful for determining a positive association.

DISCUSSION

Although evidences that FRK is involved in the regulation of pancreatic beta cells and glucose intolerance have been published, no genetic study concerning the association between *FRK* and obesity has yet been reported. In this study, we investigated whether *FRK* gene polymorphisms in Korean population are related to obesity by genotyping six SNPs, and found that only one (rs9384970) SNP was significantly associated with overweigh/obesity.

The rs6568920 (+8391G>A) is located on intron 1. The GG, GA, and AA genotype frequencies are reported to be 0.712, 0.271, and 0.017 in European, 0.711, 0.267, and 0.022 in Chinese, 0.636, 0.318, and 0.045 in Japanese, and 0.317, 0.467, and 0.217 in Sub-Saharan African, respectively (http://www.ncbi.nlm.nih.gov/SNP). In the present study, the GG, GA, and AA genotype frequencies in Korean population were 0.606, 0.329, and 0.065, respectively, which are similar to those in Japanese. The rs3756772 (+56780 A>G) is located on exon 2, and is a missense SNP (Gly122Arg) with 0.498 heterozygosity. The missense rs3756772 (Gly122Arg) is the only one SNP with known heterozygosity in the coding SNPs of *FRK* gene region (http://www.ncbi.nlm.nih.gov/SNP). The AA, AG, and GG genotype frequencies are reported to be 0.233, 0.417, and 0.350 in European, 0.622, 0.356, and 0.022 in Chinese, 0.591, 0.364, and 0.045 in Japanese, 0.017, 0.267, and 0.717 in Sub-Saharan African, and 0.053, 0474, and 0.474 in African American, respectively (http://www.ncbi.nlm.nih.gov/SNP). The AA, AG, and GG genotype frequencies in the Korean population were 0.542, 0.381, and 0.077, respectively, which are also similar to those in Japanese. FRK protein (P42685) consists of 505 amino acids and molecular mass of 58,254 Da. Amino acids from 42 to 110 comprise Src homology 3 (SH3), 116 to 208 SH2, 234 to 248 protein kinase domains, and 240 to 248 nucleotide binding region (UniProt, http://beta.uniprot.org; SwissProt, http://www.expasy.org). The SH2 domain acts as a regulatory module of intracellular signaling cascades by interacting to phosphotyrosine-containing target peptides with high affinity (http://beta.uniprot.org). However, the rs3756772 in the SH2 domain in *FRK* was not associated with obesity in Korean population (Table 2). The CC, CT, and TT genotype distributions of the rs3798234 in Korean population (0.651, 0.303, and 0.046, respectively) are close to those in Japanese. The GG, GA, and AA genotype frequencies of the rs1933739 in Korean population (0.604, 0.349, and 0.047, respectively) show patterns similar to those in Japanese. However, the genotype distributions (AA, 0.443; AG, 0.436; GG, 0.121) of the rs9400883 in Korean population are close to those in Chinese (http://www.ncbi.nlm.nih.gov/SNP, Table 2).

The rs9384970 (+68506G>A) is located on intron 2. The GG, GA, and AA genotype frequencies are reported to be 0.695, 0.288, and 0.017 in European, 0.800, 0.200, and 0.000 in Chinese, 0.750, 0.250, and 0.000 in Japanese, and 0.200, 0.600, and 0.200 in Sub-Saharan African, respectively. There is no AA genotype in 90 Chinese and 88 Japanese (http://www.ncbi.nlm.nih.gov/SNP). In the present study, the GG, GA, and AA genotype frequencies in Korean overweight/obesity subjects were 0.762, 0.203, and 0.035, respectively, while those in Korean healthy control subjects were 0.695, 0.299, and 0.006, respectively. We found the AA genotype of the rs9384970 in Korean population, and the rs9384970 was associated with overweight/obesity in the codominant model. Interestingly, AA genotype distribution in overweight/obesity group (n=7, 3.5%) was higher than those in the control group (n=1, 0.6%). Therefore, AA genotype of the rs9384970 may be a risk factor for the development of obesity in Korean population.

In conclusion, the result suggests that *FRK* gene may be associated with obesity in Korean population.

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