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A Haplotype of the *GOSR2* Gene Is Associated with Myocardial Infarction in Japanese Men

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Aims: The Golgi SNAP Receptor Complex Member 2 (*GOSR2*) gene is a Golgi-associated soluble factor attachment receptor (SNARE) protein. Some single-nucleotide polymorphisms (SNPs) in the *GOSR2* gene have been found to be associated with myocardial infarction (MI). The aim of the present study was to assess the association between the human *GOSR2* gene and MI using a haplotype-based case–control study. *Methods:* A total of 238 MI patients and 284 controls were genotyped for the five SNPs used as genetic markers for the human *GOSR2* gene (rs197932, rs3785889, rs197922, rs17608766, and rs16941382). Data were analyzed for three separate groups: the total subjects, men, and women. *Results:* The overall distribution of the haplotypes in the total subjects and the men was significantly different between the MI patients and the control subjects ($p = 0.001$, $p = 0.005$, respectively). Additionally, the frequency of the T-G-G haplotype (rs197932-rs3785889-rs197922) for men was significantly lower in the MI patients than in the control subjects ($p = 0.040$). Multiple logistic regression analysis also revealed that the frequency of the subjects with the T-G-G haplotype (homozygous and heterozygous diplotypes) was significantly lower compared with subjects without this haplotype in men after adjustment for the major confounding factors (odds ratio = 0.455, $p = 0.041$). *Conclusions:* The results of this study indicate that the T-G-G haplotype may be a protective genetic marker for MI in Japanese men.

Introduction

Coronary artery disease, including myocardial infarction (MI), is thought to be a complex multifactorial and polygenic disorder resulting from interactions between an individual's genetic makeup and various environments (Frazier *et al.*, 2005). A variety of gene variants have been shown to be associated with MI (Winkelmann and Hager, 2000; Dowaidar and Settin, 2010).

Golgi SNAP receptor complex member 2 (GOSR2) is a Golgi-associated soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein that is involved in intra-Golgi protein trafficking in multiple tissues (Lowe *et al.*, 1997). It interacts with target-localized SNAREs (t-SNAREs) to allow macromolecules, such as insulin, leptin, and angiotensinogen to move directly between Golgi compartments (Sollner *et al.*, 1993a, 1993b; Rothman, 1994).

Research at the University of California has identified the A allele in rs197922 of the *GOSR2* gene as a risk factor for MI (odds ratio [OR] = 1.17, $p = 0.032$) (Meyer *et al.*, 2009). However, the Atherosclerosis Risk in Communities Study did not find any significant differences, with the OR for the A allele in rs197922 of the *GOSR2* gene reported to be 1.08 in whites ($p = 0.066$) and 1.00 in blacks ($p = 0.951$) (Meyer *et al.*, 2009). However, at the present time, there have been no haplotype-based case–control studies that have evaluated the association between the *GOSR2* gene and MI. Therefore, the aim of the present study was to assess the associations between the human *GOSR2* gene and MI via a haplotype-based case–control study in a Japanese population.

Materials and Methods

Subjects

Subjects diagnosed with MI ($n = 238$) were recruited at the Nihon University Itabashi Hospital and at other neighboring hospitals in Tokyo. A history of MI was confirmed by the presence of two or more of the following: history of chest

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pain indicative of MI, creatine kinase, creatine kinase MB levels >3 times the upper reference limit during a post-MI follow-up, and characteristic electrocardiographic changes at the time of diagnosis (ST segment elevation >0.1 mV in at least two leads). The elevated levels of cardiac troponins were also used for the criteria of MI. Smokers were defined as current smokers or smokers who had ceased smoking. Nonsmokers were defined as subjects with no history of smoking. Diabetes mellitus was defined on the basis of the World Health Organization (WHO) criteria. Hypertension was defined as blood pressure ≥140/90 mmHg upon repeated measurements and/or the current use of antihypertensive drugs due to a history of arterial hypertension. Patients diagnosed with secondary hypertension were excluded. Hyperlipidemia was defined as a total plasma cholesterol ≥6.5 mmol or plasma triglycerides ≥2 mmol and/or the current use of lipid-lowering drugs with an established diagnosis of hyperlipidemia.

Patient age ranged from 31 to 87 years (mean ± standard deviation [SD], 61.5 ± 10.0 years). Japanese subjects ($n = 285$) were enrolled as control subjects, and ranged in age from 66 to 94 years (mean ± [SD], 77.9 ± 4.2 years). Because the mean age of the control group was older compared with the MI group, the control group was regarded as a supercontrol group. Healthy elderly subjects are more suitable than young or middle-aged subjects when determining the phenotypes of cardiovascular diseases related to aging, as many of these diseases occur late in life. Since MI is an age-influenced disease, it has been shown that a supercontrol group is better than an age-matched control group when investigating MI patients (Aoi *et al.*, 2010). Controls in this study were all members of the New Elder Citizen Movement in Japan, they all lived in Tokyo or in the suburbs of Tokyo, and they all had vascular risk factors such as hypertension, hypercholesterolemia, or diabetes mellitus, but had no history of MI. All controls were confirmed to have grade 0 on the modified Rankin Scale indicating no disability or dependence in the daily activities due to neurological disability such as stroke. Any of the participants found to have cancer or autoimmune diseases were excluded from the study group. All subjects who agreed to participate in the study were evaluated on the basis of a detailed questionnaire that provided information about coronary risk factors such as smoking habits, the presence of diabetes mellitus, or hypertension. Informed consent was obtained from each subject in accordance with the protocol approved by the Human Studies Committee of Nihon University (Yamaguchi *et al.*, 2010).

SNP selection

The human *GOSR2* gene has three transcript variants that encode three different isoforms (isoform A, isoform B, and isoform C). Isoform A of the *GOSR2* gene, which has the longest length among the three isoforms, consists of 212 amino acids and is located on chromosome 17q21. This gene consists of approximately 18.25 kilobase pairs (kbp) and contains six exons, which are separated by five introns.

There are 362 SNPs of the human *GOSR2* gene listed in the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP). In this study, we screened the data on the International HapMap Project website (http://hapmap.ncbi.nlm.nih.gov/index.html.en) for the tag SNPs of the *GOSR2* gene. SNPs with relatively high minor allele frequencies (MAFs) have been shown to be useful as genetic markers in genetic association studies. For this current genetic association study, we selected three SNPs (rs3785889, rs197932, and rs16941382) that had a MAF of > 0.1 among the Japanese population (Fig. 1). rs197932 is located 26 kbp upstream from the start codon in exon 1, whereas rs16941382 is located 25 kbp downstream from the stop codon in exon six (Fig. 1). Meanwhile, we also included rs17608766 and rs197922 from the *GOSR2* gene, as these may be associated with blood pressure and coronary heart disease, respectively (Meyer *et al.*, 2009; Ehret *et al.*, 2011).

We designated the five SNPs as SNP1 (rs197932, C_2592633_10), SNP2 (rs3785889, C_2960489_10), SNP3 (rs197922, C_2275273_10), SNP4 (rs17608766, C_33589426_10),

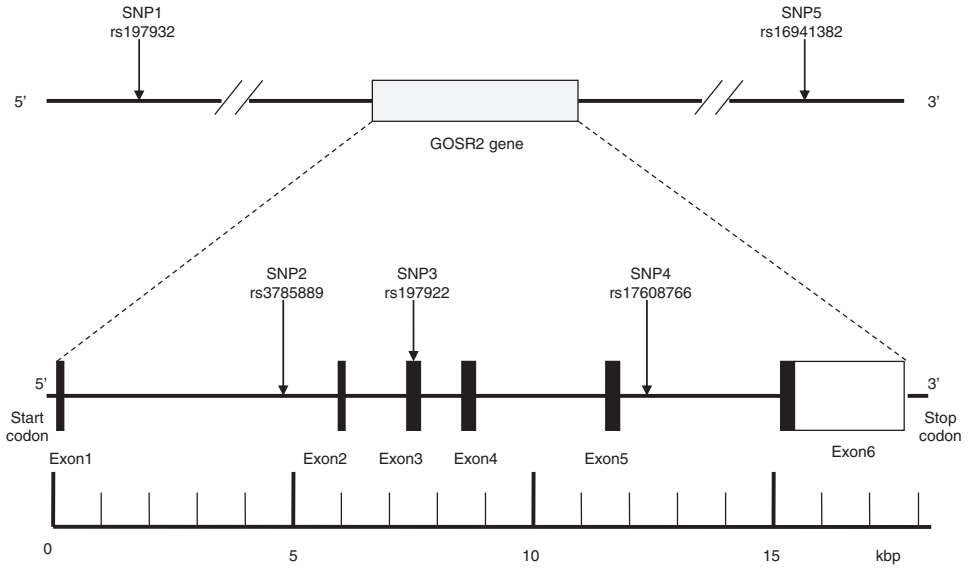

FIG. 1. Structure of the human *GOSR2* gene. The gene consists of six exons (boxes) separated by five introns (lines; intergenic regions). Filled boxes indicate the coding regions, while the white box indicated the noncoding region. Arrows indicate the locations of single-nucleotide polymorphisms (SNPs). kbp, kilobase pairs.

and SNP5 (rs16941382, C_33589395_10), which were in order of increasing the distance from the 5′ end of the gene (Fig. 1).

Genotyping

Blood samples were collected from all participants, and genomic DNA was extracted from the peripheral blood leukocytes using phenol and the chloroform extraction method (Nakayama *et al.*, 2001).

Genotyping was performed using the TaqMan SNP Genotyping Assay (Applied Biosystems) (Jiang *et al.*, 2012). The mechanism of the TaqMan SNP Genotyping method was as follows. In the first step of the 5′ nuclease assay, allele-specific fluorogenic probes were hybridized to the template. Subsequently, the 5′ nuclease activity of the Taq polymerase makes it possible for discrimination to occur during the polymerase chain reaction (PCR). The probes contain a 3′ minor groove-binding group that hybridizes to single-stranded targets, which have greater sequence specificity than the ordinary DNA probes. This reduces nonspecific probe hybridization, thereby resulting in low background fluorescence for the 5′ nuclease PCR assay (TaqMan; Applied Biosystems). Cleavage results in increased emission of a reporter dye. Each 5′ nuclease assay requires two unlabeled PCR primers and two allele-specific probes. Each probe is labeled with two reporter dyes at the 5′ end. In the present study, VIC and FAM were used as the reporter dyes. The primers and probes used in the TaqMan SNP Genotyping Assays (Applied Biosystems) were chosen based on information available at the ABI website (www.appliedbiosystems.com/AB_Home/index.htm).

PCR amplification was performed using 2.5 μL of TaqMan Universal Master Mix, No AmpErase UNG (2×) (Applied Biosystems) in a 5 μL final reaction volume, along with 2 ng DNA, 2.375 μL ultrapure water, 0.079 μL of the Tris-EDTA (TE) buffer (1×), 0.046 μL TaqMan SNP Genotyping Assay Mix (40×) containing a 331.2 nM final concentration of primers and a 73.6 nM final concentration of the probes. The thermal cycling conditions were as follows: 50°C for 2 min; 95°C for 10 min; 50 cycles of 95°C for 15 s; and 60°C for 1 min. Thermal cycling was performed using the GeneAmp 9700 system (Applied Biosystems).

Each 96-well plate contained 80 DNA samples of an unknown genotype and four reaction mixtures containing reagents, but no DNA (control). The control samples without DNA are a necessary part of the sequence detection system of the 7700 signal processing system, as outlined in the TaqMan Allelic Discrimination Guide (Applied Biosystems). The plates were read on the sequence detection system 7700 instrument with the endpoint analysis mode of the sequence detection system version 1.6.3 software package (Applied Biosystems). The genotypes were determined visually based on the dye component fluorescent emission data depicted in the X-Y scatter plot of the sequence detection system software. The genotypes were also determined automatically by the signal processing algorithms of the software. The results of each scoring method were saved in two separate output files for later comparison (Livak *et al.*, 1995).

Statistical analysis

All continuous variables were expressed as mean ± s.d. Differences in continuous variables between the MI patients

Table 1. Characteristics of Study Participants

	Total			*Men*			*Women*		
	Control subjects	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*
Number of subjects	284	238		139	192		145	46	
Age (years)	77.88 ± 4.18	61.47 ± 9.77	< 0.001[a]	77.98 ± 4.50	60.59 ± 8.94	< 0.001[a]	77.79 ± 3.86	65.11 ± 12.12	< 0.001[a]
BMI (kg/m^2)	22.64 ± 2.82	23.79 ± 3.24	< 0.001[a]	22.79 ± 2.70	24.03 ± 3.03	< 0.001[a]	22.50 ± 2.94	22.72 ± 3.95	0.836
SBP (mmHg)	135.63 ± 16.43	134.59 ± 22.03	0.339	135.57 ± 15.67	135.07 ± 20.90	0.588	135.69 ± 17.19	132.45 ± 26.65	0.305
DBP (mmHg)	78.36 ± 10.73	81.17 ± 14.78	0.064	78.72 ± 10.19	81.80 ± 14.59	0.139	78.02 ± 11.25	78.40 ± 15.49	0.981
Pulse (beats/min)	69.92 ± 10.77	75.43 ± 12.74	< 0.000[a]	68.65 ± 11.40	74.64 ± 11.21	< 0.001[a]	71.15 ± 10.00	79.08 ± 17.97	0.010[a]
Total cholesterol (mg/dl)	217.08 ± 43.80	195.91 ± 49.91	< 0.000[a]	204.09 ± 32.08	196.20 ± 45.69	0.012[a]	229.59 ± 49.68	194.62 ± 66.19	0.004[a]
Creatinine (mg/dl)	0.86 ± 0.23	1.02 ± 0.64	0.018[a]	0.96 ± 0.22	1.01 ± 0.48	0.369	0.75 ± 0.18	1.05 ± 1.11	0.811
EH (%)	20 (7.0%)	40 (16.8%)	0.001[a]	12 (8.6%)	30 (15.6%)	0.067	8 (5.5%)	10 (21.7%)	0.003[a]
Hyperlipidemia (%)	53 (18.7%)	92 (38.7%)	< 0.001[a]	12 (8.6%)	74 (38.5%)	< 0.001[a]	41 (28.3%)	18 (39.1%)	0.200
Diabetes (%)	7 (2.5%)	63 (26.5%)	< 0.001[a]	5 (3.6%)	50 (20.6%)	< 0.001[a]	2 (1.4%)	13 (28.3%)	< 0.001[a]
Drinking (%)	38 (13.4%)	65 (27.3%)	< 0.001[a]	25 (18.0%)	59 (30.7%)	0.010[a]	13 (9%)	6 (13%)	0.407
Smoking (%)	27 (9.5%)	116 (48.7%)	< 0.001[a]	21 (15.1%)	101 (52.6%)	< 0.001[a]	6 (4.1%)	15 (32.6%)	< 0.001[a]

Continuous variables are expressed as mean ± s.d. Categorical variables are expressed as percentages.
The *p* value of the continuous variables was calculated by the Mann–Whitney *U*-test.
The *p* value of the categorical variables was calculated by Fisher's exact test. [a]$p < 0.05$.
MI, myocardial infarction; BMI, body mass index; DBP, diastolic blood pressure; EH, essential hypertension; HDL, high-density lipoprotein; SBP, systolic blood pressure.

Table 2. Genotyping and Allele Distributions in Control Subjects and Patients with Myocardial Infarction

	Total			*Men*			*Women*		
Variants	*Control subjects*	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*
rs197932(SNP1)									
Genotyping									
CC	26 (9.2%)	19 (8.0%)		10 (7.2%)	12 (6.2%)		16 (11.0%)	7 (15.2%)	
CT	112 (39.4%)	99 (41.6%)		52 (37.4%)	79 (41.1%)		60 (41.4%)	20 (43.5%)	
TI′	146 (51.4%)	120 (50.4%)	0.825	77 (55.4%)	101 (52.6%)	0.782	69 (47.6%)	19 (41.3%)	0.624
Dominant model									
TI′	146 (51.4%)	120 (50.4%)		77 (55.4%)	101 (52.6%)		69 (47.6%)	19 (41.3%)	
CC+CT	138 (48.6%)	118 (49.6%)	0.861	62 (44.6%)	91 (47.3%)	0.656	76 (52.4%)	27 (58.7%)	0.500
Recessive model									
CC	26 (9.2%)	19 (8.0%)		10 (7.2%)	12 (6.2%)		16 (11.0%)	7 (15.2%)	
TT+CT	258 (90.8%)	219 (92.0%)	0.755	129 (92.8%)	180 (93.7%)	0.498	129 (89.0%)	39 (84.8%)	0.443
Allele									
C	164 (28.9%)	137 (28.8%)		72 (25.9%)	103 (26.8%)		92 (31.7%)	34 (37.0%)	
T	404 (71.1%)	339 (71.2%)	1.000	206 (74.1%)	281 (73.2%)	0.858	198 (68.3%)	58 (63.0%)	0.374
rs3785889(SNP2)									
Genotyping									
AA	11 (3.9%)	14 (5.9%)		4 (2.9%)	14 (7.3%)		7 (4.8%)	0 (0.0%)	
AG	101 (35.6%)	90 (37.8%)		55 (39.5%)	72 (37.5%)		46 (31.7%)	18 (39.1%)	
GG	172 (60.6%)	134 (56.3%)	0.446	80 (57.6%)	106 (55.2%)	0.236	92 (63.4%)	28 (60.9%)	0.280
Dominant model									
GG	172 (60.6%)	134 (56.3%)		80 (57.6%)	106 (55.2%)		92 (63.4%)	28 (60.9%)	
AA+AG	112 (39.4%)	104 (43.7%)	0.328	59 (42.5%)	86 (44.8%)	0.737	53 (36.5%)	18 (39.1%)	0.861
Recessive model									
AA	11 (3.9%)	14 (5.9%)		4 (2.9%)	14 (7.3%)		7 (4.8%)	0 (0.0%)	
GG+AG	273 (96.2%)	224 (94.1%)	0.309	135 (97.1%)	178 (92.7%)	0.091	138 (95.1%)	46 (100.0%)	0.351
Allele									
A	123 (21.7%)	118 (24.8%)		63 (22.7%)	100 (26.0%)		60 (20.7%)	18 (19.6%)	
G	445 (78.3%)	358 (75.2%)	0.239	215 (77.3%)	284 (74.0%)	0.361	230 (79.3%)	74 (80.4%)	0.883
rs197922(SNP3)									
Genotyping									
AA	70 (24.6%)	48 (20.2%)		38 (27.3%)	41 (21.4%)		32 (22.1%)	7 (15.2%)	
AG	125 (44.0%)	121 (50.8%)		56 (40.3%)	94 (49.0%)		69 (47.6%)	27 (58.7%)	
GG	89 (31.3%)	69 (29.0%)	0.266	45 (32.4%)	57 (29.7%)	0.249	44 (30.0%)	12 (26.1)	0.424
Dominant model									
AG	125 (44.0%)	121 (50.8%)		56 (40.3%)	94 (49.0%)		69 (47.6%)	27 (58.7%)	
AA+GG	159 (56.0%)	117 (49.2%)	0.135	83 (59.7%)	98 (51.0%)	0.146	76 (52.1%)	19 (41.3%)	0.236
Recessive model									
AA	70 (24.6%)	48 (20.2%)		38 (27.3%)	41 (21.4%)		32 (22.1%)	7 (15.2%)	
GG+AG	214 (75.3%)	190 (79.8%)	0.248	101 (72.7%)	151 (78.6%)	0.240	113 (77.6%)	39 (84.8%)	0.403
Allele									
A	265 (47.6%)	216 (45.5%)		132 (47.5%)	176 (45.8%)		133 (45.9%)	41 (44.6%)	
G	303 (53.3%)	259 (54.5%)	0.709	146 (52.5%)	208 (54.2%)	0.694	157 (54.1%)	51 (55.4%)	0.904
rs17608766(SNP4)									
Genotyping									
TT	284 (100.0%)	238 (100.0%)		139 (100.0%)	192 (100.0%)		145 (100.0%)	46 (100.0%)	
CT	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
CC	0 (0.0%)	0 (0.0%)	—	0 (0.0%)	0 (0.0%)	—	0 (0.0%)	0 (0.0%)	—
Dominant model									
TT	284 (100.0%)	238 (100.0%)		139 (100.0%)	192 (100.0%)		145 (100.0%)	46 (100.0%)	
CC+CT	0 (0.0%)	0 (0.0%)	—	0 (0.0%)	0 (0.0%)	—	0 (0.0%)	0 (0.0%)	—
Recessive model									
CC	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
TT+CT	284 (100.0%)	238 (100.0%)	—	139 (100.0%)	192 (100.0%)	—	145 (100.0%)	46 (100.0%)	-
Allele									
C	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
T	568 (100.0%)	476 (100.0%)	—	278 (100.0%)	384 (100.0%)	—	290 (100.0%)	92 (100.0%)	—

(*continued*)

Table 2. (Continued)

Variants	Total			Men			Women		
	Control subjects	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*	*Control subjects*	*MI patients*	p *Value*
rs16941382(SNP5)									
Genotyping									
CC	41 (14.4%)	33 (13.9%)		21 (15.1%)	28 (14.6%)		20 (13.8%)	5 (10.9%)	
CT	118 (41.5%)	114 (47.9%)		54 (38.8%)	92 (47.9%)		64 (44.1%)	22 (47.8%)	
TT	125 (44.0%)	91 (38.2%)	0.330	64 (46.0%)	72 (37.5%)	0.234	61 (42.1%)	19 (41.3%)	0.893
Dominant model									
CT	118 (41.5%)	114 (47.9%)		54 (38.8%)	92 (47.9%)		64 (44.1%)	22 (47.8%)	
CC+TT	166 (58.5%)	124 (52.1%)	0.158	85 (61.2%)	100 (52.1%)	0.117	81 (55.9%)	24 (52.2%)	0.735
Recessive model									
CC	41 (14.4%)	33 (13.9%)		21 (15.1%)	28 (14.6%)		20 (13.8%)	5 (10.9%)	
TT+CT	243 (85.6%)	205 (86.1%)	0.900	118 (84.9%)	164 (85.4%)	1.000	125 (86.2%)	41 (89.1%)	0.803
Allele									
C	200 (35.2%)	180 (37.8%)		96 (34.5%)	148 (38.5%)		104 (35.9%)	32 (34.8%)	
T	368 (64.8%)	296 (62.2%)	0.401	182 (65.5%)	236 (61.5%)	0.327	186 (64.1%)	60 (65.2%)	0.901

SNP, single-nucleotide polymorphism.

and control subjects were analyzed using the Mann–Whitney *U*-test. Differences in categorical variables were analyzed using the Fisher's exact test. Differences in distributions of genotypes and alleles between MI patients and control subjects were analyzed using the Fisher's exact test. Based on the genotype data of the genetic variations, we performed haplotype-based case–control analyses using the expectation maximization algorithm (Dempster *et al.*, 1977) and the software SNPAlyze version 3.2 (Dynacom).

When considering the results of a pairwise linkage disequilibrium (LD) analysis, a haplotype-based case–control study needs to be performed. For the analysis to succeed, all variants should be located in one haplotype block, which is indicated by a large |D′| value between each SNP (near 1). When the r^2 values are large (near 1) for the pairwise variants, one variant is not needed. The LD analysis was performed using four SNP pairs. We used |D′| values of >0.25 to assign SNP locations to one haplotype block. SNPs with an r^2 value >0.5 were selected as tagged.

In the haplotype-based case–control analysis, haplotypes with a frequency of <0.01 were excluded. The frequency distribution of the haplotypes was calculated by performing a permutation test using the bootstrap method. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors after constructing diplotypes for each subject by SNPAlyze version 3.2 (Dynacom). Statistical significance was established at $p<0.05$. Statistical analyses were performed using SPSS software for Windows, version 16.0 (SPSS).

Results

The sample size for the current study was in line with the sample size numbers that have been proposed as being appropriate in these types of the case–control studies (Olson and Wijsman, 1994).

Table 1 shows the clinical characteristics of the study participants. For total subjects, men, and women, the following values were significantly higher for the MI patients as compared to the control subjects: pulse, incidence of diabetes, and smoking. For total subjects and men, the following values were significantly higher for the MI patients as compared to the control subjects: body mass index, pulse, incidence of hyperlipidemia, diabetes, drinking and smoking. No significant difference was observed in either SBP or DBP between the MI patients and the control subjects.

Table 2 shows the distribution of the genotypes and alleles for the five SNPs. The genotype distributions for each of the SNPs were in good agreement with the predicted Hardy–Weinberg equilibrium values (data not shown). No genotype other than the TT genotype in SNP4 (rs17608766) was observed in each group. For the four other SNPs that were selected for the present study, no differences were noted for the genotype distributions, the dominant and recessive model distributions or the allele distributions in the total, male, and female subjects.

The |D′| and r^2 values for the LD patterns are presented in Table 3. All five SNPs are located in one haplotype block, as all of the |D′| were beyond 0.25. Because r^2 for SNP3-SNP5 was >0.5 in our study, this meant that SNP3 and SNP5 could not be used for constructing haplotypes simultaneously. Therefore, given that the MAF of SNP3 was larger compared with SNP5, we constructed the haplotypes using SNP1, SNP2, and SNP3.

In the haplotype-based case–control analysis, haplotypes were established through the use of different combinations of

Table 3. Pairwise Linkage Disequilibrium for Four Single-Nucleotide Polymorphisms

		|D′| *values*			
		SNP1	*SNP2*	*SNP3*	*SNP5*
r^2 values	SNP1		0.373	0.625	0.896
	SNP2	0.016		0.798	0.915
	SNP3	0.138	0.154		0.936
	SNP5	0.167	0.126	0.545	

|D′| above the diagonal and r^2 below the diagonal.
The shadowed portion indicates $|D'|>0.25$ and $r^2>0.5$.

Table 4. Haplotype Analysis in Patients with Myocardial Infarction and Control Subjects

				Overall p *Value*			*Frequency in total*			*Frequency in men*			*Frequency in women*		
Haplotype	*SNP1*	*SNP2*	*SNP3*	*Total*	*Men*	*Women*	*MI Patients*	*Control Subjects*	p *Value*	*MI Patients*	*Control Subjects*	p *Value*	*MI Patients*	*Control Subjects*	p *Value*
				0.001[a]	0.005[a]	0.551									
H1	T	G	G				0.088	0.127	0.040[a]	0.095	0.150	0.027[a]	0.059	0.106	0.133
H2	C	G	G				0.215	0.208	0.743	0.194	0.168	0.401	0.300	0.246	0.256
H3	T	A	G				0.191	0.165	0.289	0.196	0.166	0.297	0.161	0.163	0.982
H4	C	A	G				0.053	0.033	0.120	0.059	0.042	0.399	0.035	0.026	0.802
H5	T	G	A				0.431	0.400	0.289	0.437	0.406	0.436	0.411	0.396	0.779
H6	C	G	A				0.219	0.475	0.022[a]	0.018	0.050	0.021[a]	0.035	0.451	0.610
H7	T	A	A				0.000	0.187	0.002[a]	0.000	0.019	0.009[a]	0.000	0.017	0.205

Haplotype with frequencies > 0.01 were estimated using SNPAlyze software.
The *p* value was calculated by permutation test using the bootstrap method.
[a]$p < 0.05$.

the SNPs (Table 4). For the total subjects and men, the overall distribution of the haplotypes established by SNP1-SNP2-SNP3 was significantly different between the MI patients and the control subjects. The frequency of the T-G-G haplotype established by SNP1-SNP2-SNP3 in these two groups was also significantly lower for the MI patients as compared to the control subjects ($p = 0.040$). Moreover, the frequency of the C-G-A and T-A-A haplotype established by SNP1-SNP2-SNP3 in these two groups was also significantly lower for the MI patients compared to the control subjects ($p = 0.022$ and $p = 0.002$, respectively). For the women, no significant differences were found between the control subjects and MI patients.

The previously described software SNPAlyze version 3.2 was used to analyze the diplotypes in each of the subjects. Significant differences were found when a logistic regression analysis was performed using each of the confounding factors and the diplotype associated with the MI (Table 5). In men, after adjustments for BMI, diabetes mellitus, hyperlipidemia, essential hypertension, smoking and drinking, subjects with the H1 diplotype (H1 homozygote + H1 heterozygote) were found to have significantly lower chances of having a MI as compared to those without the H1 diplotype (OR = 0.455, $p = 0.041$).

Discussion

GOSR2 is a Golgi-associated soluble factor attachment receptor (SNARE) protein involved in intra-Golgi protein trafficking that is expressed in multiple tissues and organs. SNAP receptor (SNARE) complexes form a bridge of opposing membranes that promote membrane fusion within the secretory and endosomal pathways (Rothman, 1994; Hay and Scheller, 1997). The atomic structure of a single SNARE complex has been shown to consist of a highly twisted parallel bundle of four amphipathic helices, one from the vesicle protein VAMP, one from the plasma membrane protein syntaxin 1A, and two from the plasma membrane protein SNAP-25 (Poirier *et al.*, 1998; Sutton *et al.*, 1998). Most of the SNARE proteins possess either a glutamine or arginine at the deduced position. Although the precise role that SNAREs play in the specific docking and fusion of transport vesicles remains controversial, different types of experiments indicate that SNARE complexes are the core machinery for intracellular membrane fusion. Thus, the trafficking function may have a functional role in multiple cardiovascular diseases such as EH and MI.

Table 5. Odds Ratios and 95% Confidence Intervals for Each Confounding Factor and Haplotype Associated with MI in Men

Confounding factors	*Odd ratios*	*95%CI*	p *Value*
H1 haplotype (T-G-G) (homozygote + heterozygote)	0.455	0.214–0.969	0.041[a]
HL	5.765	2.727–12.188	< 0.001[a]
DM	7.368	2.658–20.420	< 0.001[a]
EH	1.291	0.537–3.106	0.568
Smoking	4.849	2.497–9.487	< 0.001[a]
Drinking	1.512	0.754–3.034	0.244
BMI	1.135	1.021–1.262	0.019[a]

[a]$p < 0.05$.
DM, diabetes mellitus; HL, hyperlipidemia; CI, confidence intervals.

Research by the University of California has identified the A allele in rs197922 of the *GOSR2* gene as a risk factor for MI (OR = 1.17, $p = 0.032$) (Meyer *et al.*, 2009). However, the Atherosclerosis Risk in Communities Study did not find any significant difference in relation to the OR for the A allele in rs197922 of the *GOSR2* gene reported to be 1.08 in whites and 1.00 in blacks ($p = 0.066$, $p = 0.951$) (Meyer *et al.*, 2009). In the present study, the frequency of the A allele in rs197922 of the *GOSR2* gene was not significantly different between the control subjects and MI patients in the Japanese population ($p = 0.709$). Our results are consistent with the findings reported for American whites and blacks. The International Consortium for Blood Pressure Genome-Wide Association Studies showed that the rs17608766 in *GOSR2* was significantly associated with blood pressure. As rs17608766 in *GOSR2* may be involved in some of the changes caused by the blood pressure in the cardiovascular system, we hypothesized that these changes could ultimately lead to MI. However, in the current study, there was only one genotype TT in the SNP4 (rs17608766) among the Japanese population. These results were consistent with the data reported for the Japanese population by the International HapMap Project.

For genes with multiple susceptibilities, an analysis based on haplotypes has advantages over an analysis based on individual SNPs, particularly when the linkage

disequilibria between the SNPs is weak (Morris and Kaplan, 2002). Our study is the first haplotype-based case–control study to investigate the association between the human *GOSR2* gene and MI in the Japanese population. In our study, we succeeded in identifying a protective haplotype T-G-G of SNP1-SNP2-SNP3 in men. Based on the diplotype and logistic regression analyses, we believe that the protective haplotype (T-G-G) is an independent protective factor for MI in Japanese men (OR = 0.455, $p = 0.041$). Interestingly, the allele that rs197922 provided to establish the T-G-G protective haplotype was the G allele. This result is consistent with the findings of the University of California, which reported that the A allele in rs197922 of the *GOSR2* gene was a risk factor for MI (Meyer *et al.*, 2009). Other case–control studies have identified some gene variants that have a gender-specific tendency for MI (Fu *et al.*, 2012). In line with these findings, our haplotype-based case–control study also showed gender specificity for the distribution of the haplotype (men only). Furthermore, we also discovered two protective haplotypes, the C-G-A and T-A-A haplotype, which were established by SNP1-SNP2-SNP3 in men ($p = 0.022$ and $p = 0.002$, respectively). However, the frequency of occurrence of these two haplotypes was too low to have any practical meaning with regard to being a genetic marker.

The mechanism by which *GOSR2* may be associated with MI remains unclear. *GOSR2* codes for a vesicular N-ethylmaleimide sensitive factor attachment protein receptor (v-SNARE) that is involved in intra-Golgi trafficking of vesicles (Hay *et al.*, 1998). v-SNAREs, such as GOSR2, interact with target-localized SNAREs (t-SNAREs) and allow the directed movement of macromolecules, such as insulin, leptin, and angiotensinogen, between Golgi compartments (Sollner, 1993a, 1993b). Due to its trafficking function, the *GOSR2* gene may be involved in cardiovascular diseases. Recently, rs17608766 in the *GOSR2* gene was reported to be negatively associated with SBP (Beta = – 0.556, $p < 0.001$) and DBP (Beta = – 0.129, $p = 0.017$) (Ehret *et al.*, 2011). This negative association with blood pressure may prevent arteriosclerosis, which suggests that some of the haplotypes in the *GOSR2* gene may be a protective factor of MI. Unfortunately, at the present time, there have yet to be any reports describing such a phenotype or a *GOSR2* knockout mouse. Thus, further studies of this variant's association with MI in animal models would be informative.

In conclusion, this is the first time that correlations between the human *GOSR2* gene and MI have been examined in the Japanese population. The present data indicate that the T-G-G haplotype of the human *GOSR2* gene might be gender-specific protective genetic markers for MI in Japanese men. Additional studies will need to be undertaken to isolate the functional mutations in the *GOSR2* gene that are responsible for regulating MI.

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Author Disclosure Statement

No competing financial interests exist.

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