Table 1 Patient characteristics

Age (years)	58 ± 15
Sex, male	27/37 (73.0 %)
BMI	21.7 ± 2.9
NYHA class I	2.7 ± 1.1
I, II, III, IV	6 (16.2 %), 9 (24.3 %), 12 (32.5 %), 10 (27.0 %)
History of heart failure	21/37 (56.8 %)
SMTD score	12.4 ± 9.5
Monomorphic VT as the index arrhythmia	23/37 (62.2 %)
Electrocardiography	
QRS duration (ms)	129 ± 39
QTc interval (ms)	493 ± 53
Echocardiography	
LVDd (mm)	67.1 ± 10.1
LVDs (mm)	56.4 ± 12.4
IVST (mm)	7.0 ± 1.1
PWT (mm)	6.9 ± 1.0
LVEF (%)	25.2 ± 8.9
Medications	
β-blockers	30/37 (81.1 %)
Class III antiarrhythmics	23/37 (59.5 %)
Spironolactone	18/37 (48.6 %)
Digitalis	19/37 (51.4 %)
Diuretics	28/37 (75.7 %)
ACEIs/ARBs	30/37 (81.1 %)

ACEIs angiotensin-converting enzyme inhibitors, *ARBs* angiotensin II receptor antagonists, *BMI* body mass index, *IVST* interventricular septum thickness, *LVDd* left ventricular diameter of end-diastole, *LVDs* left ventricular diameter of end-systole, *LVEF* left ventricular ejection fraction, *NYHA* New York Heart Association, *PWT* posterior wall thickness, *SMTD* severity of the myocardial tissue damage, *VT* ventricular tachycardia

Table 2 Univariate analysis to predict ES

	Univariate *p* value	HR	95 % CI
Age	0.08	1.04	1.00–1.10
Sex, female	0.48	1.56	0.42–4.98
BMI	0.47	0.93	0.74–1.13
NYHA class	0.04	1.92	1.02–4.09
History of heart failure	0.26	1.96	0.61–7.40
SMTD score	0.01	1.09	1.01–1.19
Monomorphic VT as an index arrhythmia	0.03	4.61	1.17–30.73
Electrocardiography			
QRS duration (ms)	0.04	1.02	1.00–1.03
QTc intervals (ms)	0.02	1.01	1.00–1.02
Echocardiography			
LVDd (mm)	0.03	1.07	1.01–1.14
LVDs (mm)	0.05	1.05	1.00–1.10
IVST (mm)	0.46	0.81	0.46–1.39
PWT (mm)	0.04	0.53	0.27–0.98
LV EF (%)	0.21	0.96	0.89–1.02
Medication			
β-blockers	0.39	0.54	0.16–2.47
Class III antiarrhythmics	0.98	0.99	0.31–3.35
Spironolactone	0.24	2.00	0.62–6.93
Digitalis	0.48	1.51	0.48–5.11
Diuretics	0.25	2.30	0.60–15.13
ACEIs/ARBs	0.82	1.18	0.31–7.71

ACEIs angiotensin-converting enzyme inhibitors, *ARB* angiotensin II receptor antagonist, *BMI* body mass index, *CI* confidential interval, *IVST* interventricular septum thickness, *HR* hazard ratio, *LVDd* left ventricular diameter of end-diastole, *LVDs* left ventricular diameter of end-systole, *LVEF* left ventricular ejection fraction, *NYHA* New York Heart Association, *PWT* posterior wall thickness, *SMTD* severity of myocardial tissue damage, *VT* ventricular tachycardia

($r^2 = 0.39$, $p < 0.0001$), and log RMS40 ($r^2 = 0.32$, $p = 0.0004$).

Discussion

The main finding of the present study was that one-third of patients with IDCM and an ICD experienced an ES during the follow-up and a SMTD score was significantly associated with the occurrence of an ES in these patients.

Incidence of ES

Twelve (32.4 %) patients experienced at least one ES episode during a mean follow-up of 43.9 ± 30.7 months (median 42.0 months, ranging from 1 to 118 months). In general, an ES occurs in 4–28 % [1, 3, 7, 8] of ICD recipients. The incidence of an ES is lower when ICDs are placed for primary prevention compared to secondary prevention. In a MADIT-II sub-study of 719 patients receiving ICDs for primary prevention, 4 % developed ESs over an average of 20.6-month period [8]. In another trial, 20 % of the patients who received ICDs for secondary prevention experienced an ES during a 31-month period [13]. Especially when focusing on IDCM patients with ICDs for secondary prevention, 28 % of the patients experienced an ES during a 32.5-month period [1]. Although the incidence seemed to be relatively higher in the present study compared to several past literatures, this discrepancy could be explained by the fact that the patients in this study were only IDCM patients with a low EF referred to our institution for the treatment of advanced CHF, and, in addition, they were followed up longer.

Table 3 Multivariate analyses adjusting for the age, sex, BMI, NYHA functional class, and LVEF

	Adjusted *p* value	HR	95 % CI
History of heart failure	0.17	0.18	0.01–2.05
SMTD score	0.02	1.09	1.01–1.19
Monomorphic VT as the index arrhythmia	0.17	3.10	0.65–24.65
Electrocardiography			
QRS duration (ms)	0.09	1.01	0.99–1.03
QTc intervals (ms)	0.07	1.01	1.00–1.02
Echocardiography			
LVDd (mm)	0.13	1.08	0.99–1.21
LVDs (mm)	0.16	1.08	0.97–1.23
IVST (mm)	0.69	1.17	0.53–2.57
PWT (mm)	0.10	0.52	0.21–1.14
Medication			
β-blockers	0.46	0.56	0.13–2.91
Class III antiarrhythmics	0.69	0.78	0.23–2.86
Spironolactone	0.49	1.52	0.46–5.51
Digitalis	0.59	1.42	0.40–5.31
Diuretics	0.17	3.05	0.66–24.20
ACEIs/ARBs	0.85	0.85	0.20–5.86

ACEIs angiotensin-converting enzyme inhibitors, *ARB* angiotensin II receptor antagonist, *BMI* body mass index, *CI* confidential interval, *IVST* interventricular septum thickness, *HR* hazard ratio, *LVDd* left ventricular diameter of end-diastole, *LVDs* left ventricular diameter of end-systole, *LVEF* left ventricular ejection fraction, *NYHA* New York Heart Association, *PWT* posterior wall thickness, *SMTD* severity of myocardial tissue damage, *VT* ventricular tachycardia

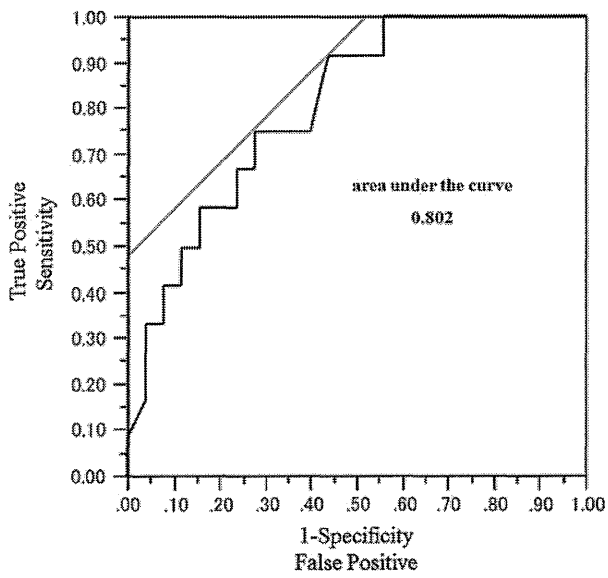

Fig. 2 A sensitivity–specificity analysis using receiver operating characteristics (ROC) curves. A cut-off value of the SMTD score set at 10 optimized the capability of predicting an ES

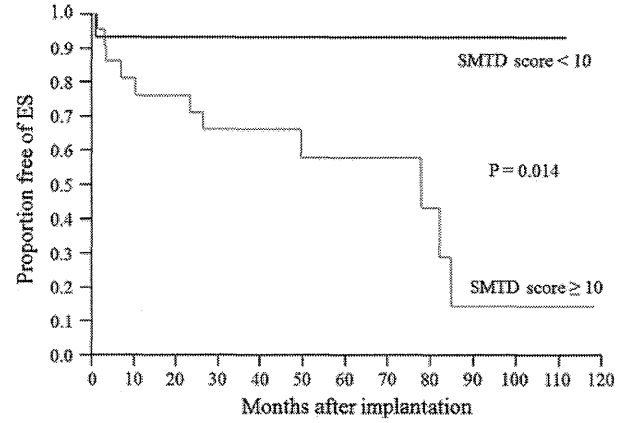

Fig. 3 Kaplan–Meier curves of the freedom from ES events between the groups with SMTD scores of ≥10 and <10 ms. The risk of an ES occurrence was significantly higher among the IDCM patients with SMTD scores of ≥10 than in those with scores of <10 ($p = 0.014$). *SMTD* severity of the myocardial tissue damage

SMTD score as a predictor of an ES

A higher SMTD score remained as a significant predictor of an ES in the multivariate analysis. The risk of an ES increased by 9 % for each additional 1-point increase in the SMTD score ($p = 0.02$, HR 1.09/1 increase in the score, CI 1.01–1.19). The optimized cut-off SMTD score determined from the ROC curves for differentiating patients with and without an ES was 10 in this study. At this cut-off score, the SMTD predicted an ES with 92 % sensitivity, 56 % specificity, and positive and negative predictive values of 52 % and 94 %.

As far as we know, this is the first report to describe an association between the SMTD and ES occurrence in IDCM patients with an ICD. However, several reports have indicated the significance of the SMTD as a predictor of ventricular tachyarrhythmias or the prognosis in patients with an old myocardial infarction (OMI). De Sutter et al. [14] reported that the extent of scarring determined by myocardial perfusion imaging can separate patients with coronary artery disease into high- and low-risk groups in terms of recurrent ventricular arrhythmias and cardiac hospitalization after the ICD implantation. Nishisato et al. [15] demonstrated that impairment of cardiac sympathetic innervation and myocardial perfusion is related to lethal arrhythmic events leading to sudden death, and the combined assessment of these might be useful for identifying patients who need a prophylactic ICD. Morishima et al. [16] showed that perfusion volume defects determined by Tc-99m tetrofosmin scintigraphy in patients with an OMI is a pivotal predictor of future lethal arrhythmic events and sudden cardiac death. They also suggest the mechanism of arrhythmogenicity using signal-averaged ECGs. A larger

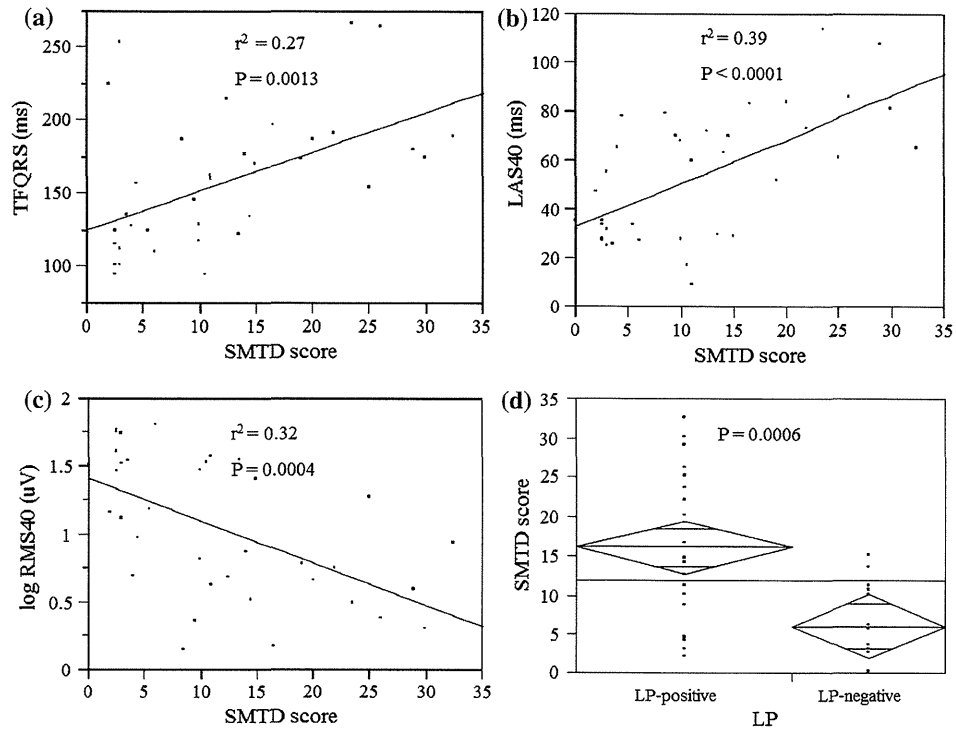

Fig. 4 Relationship between the SMTD score and late potentials. The SMTD score was significantly correlated with the **a** TFQRS ($r^2 = 0.27$; $p = 0.0013$), **b** LAS40 ($r^2 = 0.39$; $p < 0.0001$), and **c** logRMS40 ($r^2 = 0.32$; $p = 0.0004$). **d** Moreover, the SMTD score was significantly larger in the patients with than in those without late potentials (16.1 ± 9.4 and 6.1 ± 4.7, respectively; $p = 0.0006$). *LAS40* duration of the terminal low amplitude signals of <40 μV in the terminal-filtered QRS complex, *LP* late potentials, *SMTD* severity of the myocardial tissue damage, *TFQRS* total filtered QRS duration, *RMS40* root-mean-square voltage of the terminal 40 ms of the filtered QRS complex

area of the volume defect is highly correlated with an increasing prevalence of LPs, which suggests the existence of a greater arrhythmogenic substrate increasing the risk of ventricular arrhythmias. Our findings from the SAECG also suggested the association between the LPs and SMTD score (Fig. 4). All three indices of the LPs were significantly correlated with the SMTD score from the MPS ^{99m}Tc MIBI scintigraphy. Thus, lethal ventricular arrhythmias including VT and VF are considered to arise on the basis of an arrhythmogenic substrate comprised of necrotic, fibrotic or degenerative myocardial tissue. However, other modulating factors such as ischemia, drugs, heart electrolytes, autonomic nerve activity and stress, or triggers such as premature ventricular contractions might be also taken into consideration. An arrhythmogenic substrate can be visualized as a defect area on the MPS [17]. We previously reported the significance of the SAECG in predicting an ES [9]. However, the data of the SAECG were, unfortunately, not obtained in all patients, and the strong correlation of these SAECG parameters and the SMTD score made it impossible to reveal the independent significance of each other in the small study population.

Other predictors of an ES

Although the clinical value of LVEF in predicting an ES has not fully been elucidated, several reports have revealed the significant association between LVEF and an ES [1, 3, 4, 7, 18–21]. Bausch et al. [1] revealed that a low LVEF (<40 %) is one of the best predictors of an ES in patients with IDCM, and Lunati et al. [18] showed that an LVEF <25 % is significantly associated with VT/VF clusters in patients with a biventricular ICD. However, Narasimhan et al. [20] demonstrated that the role of the LVEF is not always a strong predictor and is limitedly useful in patients with coronary artery disease. In the present study, the LVEF was not significantly associated with the occurrence of an ES when analyzed with the SMTD by a multivariate analysis. The discrepancies between several studies and the present study regarding the LVEF might be explained by the difference in the population size, severity or type of underlying disease, type of device [ICD or cardiac resychronization therapy defibrillator (CRT-D)], or the definition of an ES [1, 3, 4, 7, 8, 18–21].

Monomorphic VT is still controversial as a predictor of an ES. However, several studies support its significance in nonischemic dilated cardiomyopathy [1, 18]. While the mechanism responsible for the clustering of tachycardia is uncertain, clustering may be facilitated by the presence of ventricular scar [22, 23], and this mechanism is theoretically acceptable. In the present study, monomorphic VT was one of the predictors of an ES in the univariate analysis, but it did not remain so in the multivariate analysis. The small population of our study design may affect the results in this point.

Clinical implications

Several investigators have examined the prognostic significance of VT/VF episodes, and most have found that the development of electrical clusters or storms identifies ICD recipients with a transiently higher risk of death [18]. Stratifying the risk of an ES with the SMTD score enables us to achieve the appropriate choice of medication and careful follow-up in specific patients. In addition, recent reports revealed the possibility of empirical ablation techniques for substrate modification to prevent or reduce VT/VF episodes [24, 25]. However, further work up should be required in this field.

Study limitations

Several limitations have to be considered. First, this is a retrospective observational study with a small population in a single center. Second, echocardiographic parameters (LVDd, LVDs, and LVEF) were used instead of those determined by ECG-gated myocardial perfusion SPECT (EF, EDV, and EDV) to assess LV function, because the precise data of gating were not able to be acquired from all patients because of the frequent ventricular or atrial arrhythmias during the examination. Third, CRTD should have been used in our study population to reduce the occurrence of an ES, it was not available in Japan at that time.

Conclusion

SMTD score assessed by MPS has a strong correlation to the late potentials and higher SMTD score may increase the risk of ES among patients with IDCM and an ICD.

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RESEARCH ARTICLE

Human Mutation

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CDH13 Gene Coding T-Cadherin Influences Variations in Plasma Adiponectin Levels in the Japanese Population

Hiroko Morisaki,[1] Itaru Yamanaka,[1] Naoharu Iwai,[2] Yoshihiro Miyamoto,[3,4] Yoshihiro Kokubo,[4] Tomonori Okamura,[4] Akira Okayama,[4] and Takayuki Morisaki[1,5]*

[1]*Departments of Bioscience and Genetics;* [2]*Genomic Medicine, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan;* [3]*Departments of Atherosclerosis, Metabolism and Clinical Nutrition;* [4]*Preventive Cardiology, National Cerebral and Cardiovascular Center, Osaka, Japan;* [5]*Department of Molecular Pathophysiology, Osaka University Graduate School of Pharmaceutical Sciences, Osaka, Japan*

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ABSTRACT: Adiponectin is most abundantly expressed in adipose tissue and well known to play an important role in metabolic regulation. Several studies have attempted to identify the genetic determinants of metabolic syndrome (MetS), though no study has revealed a *cis*- or *trans*-single nucleotide polymorphism (SNP) that affects plasma adiponectin levels, except the adiponectin structure gene and genes encoding adiponectin-regulatory proteins. We performed a genome-wide association study in regards to plasma adiponectin concentrations in 3,310 Japanese subjects. We identified the strongest statistically associated SNP (rs4783244) with adiponectin levels ($P = 3.8 \times 10^{-19}$) in the first intron of *CDH13* (T-cadherin) gene in a 30-kb haplotype block covering the promoter region to first intron. In addition, rs12051272 SNP genotypes in linkage disequilibrium with rs4783244 were found to be more significantly associated with adiponectin levels ($P = 9.5 \times 10^{-20}$) and specifically with the levels of high-molecular weight (HMW) adiponectin, a subtype form associated with parameters related to glucose metabolism. Our results did show more significant association with adiponectin levels than rs12444338 (in *CDH13*) SNP genotypes reported recently. We suggest that the phenotype-affecting haplotype tagged by rs12051272 SNP would affect the plasma adiponectin levels and that we have to take the CDH13 genotype into account before considering the functional relevance of the adiponectin level.
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KEY WORDS: GWAS; CDH13; adiponectin; SNP

Introduction

Adiponectin is one of the most abundant gene products expressed in adipose tissue [Maeda et al., 1996]. It is also well known to play an important role in metabolic regulation affecting obesity, insulin sensitivity, or atherosclerosis [Yamauchi et al., 2001]. Plasma adiponectin levels are known to be correlated with body mass index (BMI), type 2 diabetes mellitus (T2DM), or even coronary artery disease [Hotta et al., 2000]. Several studies have also shown that adiponectin plays many metabolic effects including antidiabetic, antiatherosclerotic, or anti-inflammatory action [Matsuzawa et al., 2004]. Also, it is suggested to affect the relationship between obesity and insulin resistance or T2DM [Kern et al., 2003]. Production of adiponectin and plasma adiponectin concentrations was known to be regulated by complexed mechanisms [Yu and Ginsberg, 2005]. For example, its expression is increased by leanness, adrenalectomy, insulin-like growth factor 1, ionomycin, or thiazolidinediones, while it is decreased by obesity, tumor necrosis factor-α, glucocorticoids, β-adrenergic agonists, or cyclic AMP. In addition, genetic factors are also suggested to regulate adiponectin concentration as shown by the family study [Menzaghi et al., 2007] or several genome-wide linkage scans [Yang and Chuang, 2006]. Several candidate genes include the adiponectin structural gene (*ADIPOQ*; MIM# 605441) as well as the genes encoding adiponectin–regulatory proteins have been postulated to influence the adiponectin concentration [Ntalla et al., 2009], though the role of genetic variants regulating adiponectin function on insulin resistance, T2DM, or coronary artery disease, has not been clearly determined.

Recently, the development of low-cost, high-throughput genotyping technology made it possible to identify common genetic variants influencing health outcomes on a genome-wide scale. Several studies were performed to identify the genetic determinants of metabolic syndrome (MetS) and related traits. In one study, a comprehensive assessment of the genetic determinants of adiponectin levels was performed on a genome-wide basis in northern and western European population in addition to genome-wide linkage and association analyses, and the genetic influences on plasma levels of adiponectin were evaluated [Ling et al., 2009].

Here, to identify genes influencing variation in plasma adiponectin levels, we performed a genome-wide association study on plasma adiponectin concentration in subjects recruited in Suita, Osaka, Japan.

Materials and Methods

Suita Study

The Suita study was initiated as a cohort study for cardiovascular diseases of urban residents of Japanese in 1989. The details of this study were described elsewhere [Iwai et al., 2002]. Data from 5,098

Additional Supporting Information may be found in the online version of this article.

*Correspondence to: Takayuki Morisaki, National Cerebral and Cardiovascular Center Research Institute, Bioscience and Genetics, 5-7-1 Fujishirodai, Suita, Osaka, 565-8565, Japan. E-mail: morisaki@ri.ncvc.go.jp

participants (2,404 men and 2,694 women) were initially included in the analysis. DNA samples were prepared from 3,310 participants (1,527 men and 1,783 women) after informed consent was obtained. This cohort study was approved by the ethics committee of the National Cardiovascular Center. Subjects received a physical exam during which height, weight, and waist circumference were measured according to a standardized protocol. Blood samples were collected after a 12-hr fast. Plasma adiponectin level and other blood chemical levels including serum total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglyceride (TG), glucose, and insulin were measured. Hypertension was defined as either a systolic blood pressure (SBP) ≥140 mm Hg, a diastolic blood pressure (DBP) ≥90 mm Hg, or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥7.0 mmol/L (126 mg/dl), the use of antidiabetic agents, or both. MetS was defined using modified NCEP-ATP III criteria [Heng et al., 2006] based on the International Obesity Task Force central obesity criteria for Asia [Kanazawa et al., 2002].

Yahaba Study

The Yahaba study was initiated as a cohort study for cardiovascular diseases of rural residents in 2007. DNA samples were prepared from 172 participants (69 men and 103 women) after informed consent was obtained. This cohort study was also approved by the ethics committee of the National Cardiovascular Center. Blood samples were collected as the Suita study. The samples were also used for the measurement of the same parameters performed in the Suita study.

Adiponectin Measurement

Plasma adiponectin level was measured using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). Prior to genetic analyses, mean levels of adiponectin and other traits were compared between groups using linear regression in SAS version 9 (SAS Institute, Cary, NC).

Association Study

The association study was initially carried out to identify specific single nucleotide polymorphisms (SNPs) associated with variation in log-transformed adiponectin levels in the Suita Study. Genotyping was initially performed using the Illumina 550K chip (Illumina, San Diego, CA). TaqMan system (Life Technologies, Carlsbad, CA) was then used for further genotyping of the individual SNPs. Exclusion criteria of SNP genotypes for this study were minor allele frequency (MAF) <0.1, *P*(Hardy–Weinberg equilibrium) <0.05, or typing rate <0.98. The genome-wide SNP association analysis for adiponectin was performed in a simple linear regression and additive genetic model without adjustment. In addition to reporting marker-wise statistical test results, genome-wide levels of statistical significance were calculated by applying a Bonferroni correction. Linkage disequilibrium structure was evaluated at selected locus regions using the Haploview software (Broad Institute, Cambridge, MA). For further genotyped individual SNPs, association analysis was performed in a multiple linear regression model with adjustment for age, sex, and BMI.

Results

The statistical analysis in the initial stage (Suita-1) was performed with the data from 842 individuals of Suita study. Based on the exclusion criteria of SNP genotypes, 348,622 SNPs were analyzed. Figure 1 shows genome-wide association with plasma adiponectin as well as a plot of the *P*-values of each SNP according to its physical location encompassing *CDH13* gene (MIM# 601364) region in the initial screening of samples of Suita study. In this analysis, genomic inflation factor (based on median chi-squared) was 1 and cutoff *P*-value after applying a Bonferroni correction was 1.43×10^{-7}. Seven SNPs (four SNPs in choromosome 11 and three SNPs in chromosome 16) were significantly associated with plasma adiponectin. Since these SNPs in chromosome 16 (rs4783244:G>T, rs9940180:C>T, rs7193788:A>G) were located in *CDH13* gene but SNPs in chromosome 11 (rs563272:C>T at 115512749, rs483058:A>G at 115513725, rs7125373:A>G at 115532483, rs1621764:C>T at 115538977) were not located within or near the known genes, we concentrated SNPs located in chromosome 16 in this study. We also evaluated the linkage disequilibrium plots of these SNPs in chromosome 16. The strongest statistically associated SNP (rs4783244) lay in the first intron of *CDH13* gene in a 30-kb haplotype block covering the promoter region to first intron. Haplotype analysis using seven SNPs in this block (rs16957844:A>C, rs3844412:C>T, rs3865186:C>T, rs9940180, rs7193788, rs4783244, rs8047711:A>G) revealed that the haplotype with the strong evidence of association was indeed tagged by a single tag SNP, rs4783244 (Supp. Table S1). Since the selection of tag SNPs in Illumina Infinium II assay was based on the haplotype data of Caucasian population, we next evaluated the haplotype block structure in Japanese population using genotype data available from the international HapMap project. Although the extent of haplotype block of this region in Japanese was similar with that in Caucasians based on the calculated linkage disequilibrium (LD) measure r^2 (Supp. Fig. S1), major haplotype structure consisting the haplotype block including rs4783244 was quite different, suggesting we should reassess the tag SNP selection using Japanese set of SNP data. All HapMap SNPs available in 33-kb region bounded by SNPs rs2318177:C>T and rs8045889:C>T that covers the 30-kb haplotype block, were screened by LD measure—presenting the $r^2 < 0.7$ with all tag SNPs in Illumina assay system. Seven SNPs (rs11646213:A>T, rs12051272:G>T, rs16957848:C>T, rs3852729:A>C, rs3865183:A>G, rs6565051:A>G, rs8060461:A>G) met these criteria. Of these, six SNPs (rs11646213, rs16957848, rs3852729, rs3865183, rs6565051, rs8060461) were excluded since they showed stronger r^2 with other SNPs presented in Illumina assay system than with rs4783244 (Supp. Table S2). So, the TaqMan probe set predesigned for the remained rs12051272, which had been excluded from Illumina assay system because of a low MAF in Caucasian population (Supp. Table S2), was obtained and genotyped in the same Japanese population. Interestingly, the rs12051272 SNP presented even stronger association with plasma adiponectin levels than rs4783244 ($P = 2.6 \times 10^{-13}$ for unadjusted, and 9.5×10^{-20} adjusted for sex, age, and BMI) in this initial stage study (Suita-1).

Next we genotyped these two SNPs, rs4783244 and rs12051272, in the whole set of Suita Study subjects using a TaqMan PCR method (Table 1). We intensively included the samples from the first screening to measure the typing discrepancy between two methods. The replicate error rate between two methods in rs4783244 was 0.59%. Those five subjects with ambiguous data were accordingly excluded from the further analysis.

The statistical analysis in the second stage (Suita-2) was then conducted with the data from remaining 2,468 Suita study participants left out from the first screening (Table 1). The basic characteristics of these two groups are similar as shown in Table 2. The mean age was older in the second group as expected since the aged participants were excluded from the first screening. Accordingly, the mean adiponectin level, which was known to be affected by age, was higher

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Table 1. Results of Genotyping Two SNPs, rs4783244 and rs12051272, in the *CDH13* Gene, in Suita-1, Suita-2, or Yahaba

Set				Suita-1					Suita-2					Yahaba				
Number(M/F)				373/464					1144/1311					68/101				
						P-value					*P*-value					*P*-value		
SNP	MAF			Mean	SD	Unadj	a_1s adj	a_1s,B adj	Mean	SD	Unadj	a_1s adj	a_1s,B adj	Mean	SD	Unadj	a_1s adj	a_1s,B adj
rs4783244	0.31	Female	11	6.26	4.96	3.56×10^{-8}	1.51×10^{-8}	8.98×10^{-9}	8.00	5.57	2.26×10^{-9}	2.09×10^{-8}	2.35×10^{-10}	9.53	4.60	0.32	0.33	0.30
			12	8.06	5.42				8.60	5.71				11.4	6.95			
			22	9.78	6.49				10.0	6.38				11.8	8.82			
		Male	11	3.60	1.57	5.17×10^{-9}	1.63×10^{-9}	1.11×10^{-12}	4.72	3.62	8.14×10^{-10}	4.85×10^{-11}	6.30×10^{-13}	4.91	5.22	5.88×10^{-5}	6.33×10^{-5}	7.32×10^{-5}
			12	5.12	3.23				5.79	4.54				6.03	5.29			
			22	6.12	4.34				6.64	4.98				10.0	4.58			
		Combined	11	4.80	3.60	7.87×10^{-13}	1.55×10^{-16}	3.83×10^{-19}	6.30	5.25	4.24×10^{-15}	2.31×10^{-17}	1.60×10^{-21}	7.31	6.33	0.0031	0.0007	0.0006
			12	6.65	4.89				7.15	5.58				9.06	7.69			
			22	7.87	6.06				8.26	6.25				11.0	6.96			
rs12051272	0.31	Female	11	5.83	4.28	3.82×10^{-9}	1.63×10^{-9}	1.45×10^{-9}	7.94	5.55	1.53×10^{-9}	1.18×10^{-8}	1.49×10^{-10}	9.53	4.60	0.45	0.45	0.42
			12	8.12	5.40				8.58	5.78				11.6	6.92			
			22	9.70	6.60				10.0	6.33				11.6	8.78			
		Male	11	3.65	1.65	8.81×10^{-9}	3.22×10^{-9}	2.30×10^{-12}	4.68	3.62	7.60×10^{-10}	4.39×10^{-11}	5.32×10^{-13}	4.91	5.22	5.88×10^{-5}	6.33×10^{-5}	7.32×10^{-5}
			12	5.08	3.23				5.78	4.53				6.03	5.29			
			22	6.11	4.31				6.63	4.95				10.0	4.58			
		Combined	11	4.65	3.23	2.61×10^{-13}	2.82×10^{-17}	9.46×10^{-20}	6.25	5.24	2.61×10^{-15}	1.03×10^{-17}	8.07×10^{-22}	7.31	6.33	0.0047	0.0013	0.0011
			12	6.68	4.93				7.14	5.61				9.12	7.77			
			22	7.85	6.08				8.25	6.21				10.9	6.96			

M, male; F, female; MAF, minor allele frequency; 1, minor allele; 2, major allele; unadj, unadjusted; a_1s adj, age and sex adjusted; a_1s,B adj, age, sex, and BMI adjusted.

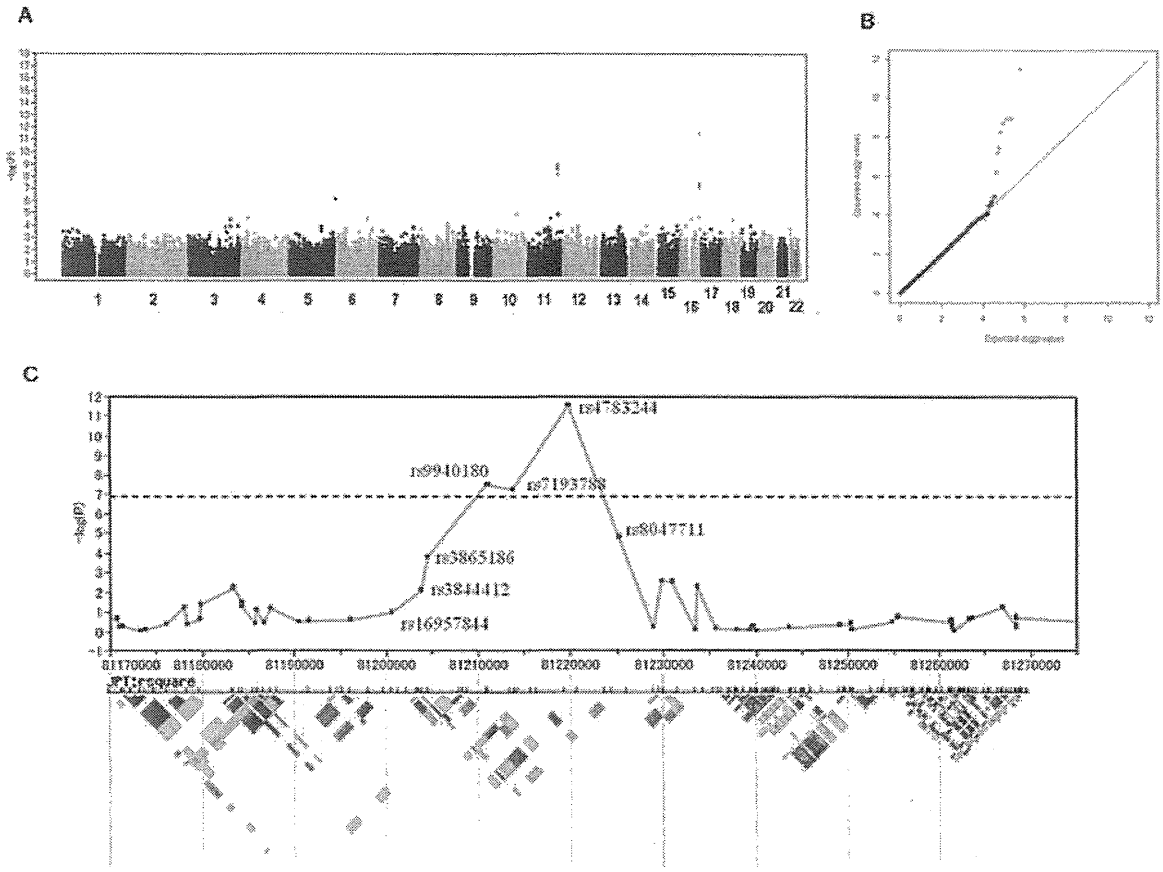

Figure 1. Genome-wide association with plasma adiponectin in Suita population. **A:** Manhattan plot. **B:** PP plot. **C:** *P*-values of each SNPs according to its physical location encompassing *CDH13* gene region were shown.

in the second group ($P < 0.001$). But the intergroup difference in plasma adiponectin levels was not significant after adjustment for age and sex ($P = 0.23$). Using the second group, the two SNPs examined showed again statistically significant association with log-transformed plasma adiponectin levels ($P = 4.2 \times 10^{-15}$ and 2.6×10^{-15} for rs4783244 and rs12051272, respectively). The association was even more significant after adjustment for sex, age, and BMI ($P = 1.6 \times 10^{-21}$ and 8.1×10^{-22}). The association of rs12051272 alleles was slightly more significant than that of rs4783244, same as the result for the first group ($P = 3.8 \times 10^{-19}$ and 9.5×10^{-20}, respectively after adjustment for sex, age, and BMI). The evidence of association was strongest ($P = 4.4 \times 10^{-37}$ and 6.9×10^{-38}) when data from two groups were combined (Suita-1 + Suita-2) and adjusted for sex, age, and BMI (Supp. Table S3). Finally, pairwise LD (r^2) between rs4783244 and rs12051272 in Suita study group was 0.97. From these data, we concluded that the haplotype tagged by rs12051272 was most responsible for the association with plasma adiponectin levels.

Table 2. The Basic Characteristics of Groups for the Study

	Suita-1	Suita-2	Yahaba	
Number	842	2468	172	
Female sex percent	55.6	53.3	59.9	
Age	58.2 (7.0)	66.7 (11.3)	62.2 (6.6)	b,c
BMI (kg/m^2)	22.8 (2.9)	22.9 (3.2)	24.3 (3.1)	c
Waist/hip ratio	0.884 (0.046)	0.907 (0.052)	0.913 (0.073)	b,c
Adiponectin (μg/dl)	7.0 (5.5)	7.5 (5.9)	9.8 (7.6)	b,c
TC (mg/dl)[a]	211.5 (32.5)	207.4 (32.3)	211.0 (32.8)	b
LDLc (mg/dl)	128.4 (31.1)	126.1 (29.5)	131.6 (32.0)	
HDLc (mg/dl)	61.5 (15.6)	59.9 (15.6)	60.3 (14.0)	b
TG (mg/dl)	92.7 (65.7)	93.4 (61.6)	100.9 (58.9)	c
SBP (mm Hg)[a]	123.1 (17.3)	131.4 (19.6)	122.8 (20.1)	b,c
DBP (mm Hg)	77.4 (10.0)	78.0 (10.0)	73.0 (12.0)	c
Fasting insulin (μU/ml)	4.4 (3.3)	4.8 (3.8)	5.5 (4.9)	b,c
Fasting glucose (mg/dl)[a]	94.8 (14.4)	99.2 (18.8)	88.1 (14.2)	b,c

All measures were shown in mean (SD).
[a]Variables were log tranformed for initial calculation of mean and SD. Values in table were shown as untransformed values.
[b]Statistically significant difference.
[c]Statistically significant difference between Suita (1 + 2) and Yahaba cohort.

Since the first and second groups were derived from the single cohort, though they did not overlap each other, we next conducted an additional analysis using the data from Yahaba study, another residential cohort study originated in northern part of Japan to confirm our results from Suita study. The population characteristics of these two studies were different in several points. The cohort in Suita study are residents in the urban area of Osaka, the second largest city of Japan, and most of the residents originated in western part of Japan, and settled down about half a century ago. In contrast, Yahaba is a small town located in the rural area of northern part of Japan and its

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Table 3. SNP Genotypes in CDH13 and Adiponectin Types in Yahaba

			HMW adiponectin						Non-HMW adiponectin				
						Additive *P*-value					Additive *P*-value		
SNP	MAF		Genotype	Mean	SD	Unadj	a,s adj	a,s,B adj	Mean	SD	Unadj	a,s adj	a,s,B adj
rs4783244	0.30	Female	22	7.43	6.96	0.46	0.46	0.42	4.67	2.19	0.15	0.15	0.13
			12	7.36	5.15				4.26	1.84			
			11	6.00	3.15				3.72	1.45			
		Male	22	6.38	3.48	4.10×10^{-5}	4.50×10^{-5}	3.80×10^{-5}	3.69	1.38	0.24	0.23	0.38
			12	3.04	3.39				3.31	1.77			
			11	3.33	2.36				3.05	1.10			
		Combined	22	6.95	5.40	0.0071	0.001	0.001	4.24	1.93	0.113	0.062	0.056
			12	5.35	5.94				3.92	1.86			
			11	4.86	3.42				3.48	1.34			
rs12051272	0.30	Female	22	7.33	6.86	0.56	0.56	0.52	4.61	2.21	0.22	0.22	0.20
			12	7.46	5.19				4.32	1.82			
			11	6.00	3.15				3.72	1.45			
		Male	22	6.38	3.48	4.13×10^{-5}	4.50×10^{-5}	3.83×10^{-5}	3.69	1.38	0.24	0.23	0.38
			12	3.04	3.39				3.31	1.77			
			11	3.33	2.36				3.05	1.10			
		Combined	22	6.91	5.36	0.009	0.002	0.002	4.21	1.94	0.156	0.095	0.089
			12	5.37	6.02				3.95	1.85			
			11	4.86	3.42				3.48	1.34			

MAF, minor allele frequency; 1, minor allele; 2, major allele; unadj, unadjusted; a,sadj, age and sex adjusted; a,s,B adj, age, sex, and BMI adjusted.

residents were mostly indigenous since 1800s. The rs4783244 and rs12051272 SNPs were genotyped using the TaqMan PCR method as described earlier. Although the sample size in Yahaba was smaller compared to the previous two studies, we found a statistically significant association ($P = 0.0006$ and 0.0011, respectively) after male and female were combined. Here, female samples in Yahaba did not show significant association between adiponectin levels and either SNP genotype under any genetic model including a dominant model (data not shown), while the MAF in Yahaba was 0.31 and not different from that for Suita study. This might be due to small number of Yahaba study or characteristics of Yahaba female. *CDH13* gene codes for T-cadherin that had been identified as a receptor specific for high-molecular weight (HMW) adiponectin [Hug et al., 2004]. We next examined if the association was specific for the levels of HMW adiponectin and not for that of low-molecular weight (LMW) adiponectin using data from Yahaba study. As expected, rs12051272 SNP genotypes were significantly associated only with the levels of HMW adiponectin ($P = 0.0018$ after adjustment for sex, age, and BMI) and not with that of other types of adiponectin ($P = 0.09$) (Table 3). However, there was weaker association between SNP genotype and HMW adiponectin in female than in male partly because of sex difference in plasma adiponectin concentrations (female: 11.4 μg/dl, male: 7.7 μg/dl, $P = 9 \times 10^{-6}$). Since several SNPs or mutant within the *ADIPOQ* gene, which codes for adiponectin, had been reported to be associated with plasma adiponectin levels previously, we genotyped these SNPs additionally. These included rs2241766:G>T (Gly15:exon1), rs1501299:A>C (intron1), rs710445:A>G (promoter), and Ile164Thr (nonsynonymous substitution). They indeed showed a weak but statistically significant association with adiponectin levels in our Suita study subjects ($P = 0.00093$, 0.32, 0.0001, 2.2×10^{-25}, respectively), but the magnitude of significance was smaller compared that of *CDH13* SNP rs12051272. The effect size presented in eta-squared for rs12051272, rs2241766, rs1501299, rs710445, and Ile164Thr was 3.28%, 0.27%, 0.02%, 0.37%, and 2.62%, respectively. The beta-coefficient per allele for them was 27.7%, 8.0%, 2.4%, 8.7%, and 127.5%, respectively. Ile164Thr mutant showed the largest beta-coefficient, but the MAF was very low (0.8%), resulting in smaller eta value than that of rs12051272 (Table 4).

Since T-cadherin has been shown to bind low density lipoprotein (LDL) cholesterol [Resink et al., 1999], we next analyzed the association between rs12051272 genotypes and the levels of LDL cholesterol using the data from whole Suita study participants. The rs12051272 genotypes showed no significant association with serum LDL cholesterol levels ($P = 0.19$), as well as with total or HDL cholesterol levels ($P = 0.46$ and 0.16, respectively). Plasma adiponectin levels also have been shown independently related with many obesity-related phenotypes, including insulin resistance [Lindsay et al., 2002; Snehalatha et al., 2003], the levels of fasting insulin, fasting glucose, and fasting TG. Since they showed a significant association also in our study, we next evaluated the association between those phenotypes and the rs12051272 genotypes (Table 5A). Interestingly, although we failed to find any statistical association between them in simple regression analysis adjusted for age and sex, we found a significant association when log-transformed plasma adiponectin level was included as an explanatory variable. The partial correlation coefficient between plasma adiponectin levels and homeostasis model assessment-estimated insulin resistance (HOMA-IR), the levels of fasting insulin, fasting glucose, and fasting TG in Suita study population was −0.39 ($P = 5.00 \times 10^{-102}$), −0.38 ($P = 7.04 \times 10^{-94}$), −0.23 ($P = 1.77 \times 10^{-34}$), and −0.36 ($P = 4.48 \times 10^{-85}$), respectively, while that of plasma adiponectin levels and rs12051272 genotypes (additive model) was −0.24 ($P = 2.0 \times 10^{-34}$). Similarly, that of

Table 4. Effect Size of SNPs on Serum Adiponectin Levels

		Serum adiponectin (μg/ml), mean (SD)				Eta squared			Beta coefficient		
	Allele1 f	Allele 1	Both	Allele2	*P* value (a)	SNP (a)	Age	Sex	SNP (p ad)	Age	Sex (p sex)
rs12051272	0.688	8.01 (0.10)	6.87 (0.09)	5.75 (0.17)	2.0×10^{-31}	3.28%	5.77%	15.35%	27.7%	24.2%	79.1%
rs4783244	0.684	8.03 (0.11)	6.88 (0.09)	5.84 (0.17)	1.7×10^{-30}	3.17%	5.71%	15.43%	27.1%	24.1%	79.3%
rs2241766	0.295	7.66 (0.24)	7.45 (0.11)	7.05 (0.09)	9.3×10^{-4}	0.27%	5.82%	15.30%	8.0%	24.3%	79.0%
rs1501299	0.277	7.18 (0.23)	7.2 (0.11)	7.33 (0.09)	0.32	0.02%	5.93%	15.14%	2.4%	24.5%	78.6%
rs266729	0.748	7.4 (0.09)	7.16 (0.11)	6.77 (0.24)	0.009	0.17%	5.84%	15.29%	6.6%	24.3%	79.0%
rs710445	0.412	7.72 (0.17)	7.33 (0.10)	6.97 (0.11)	1.0×10^{-4}	0.37%	5.84%	15.14%	8.7%	24.3%	78.6%
rs822395	0.063	5.02 (0.86)	7.01 (0.19)	7.31 (0.07)	0.029	0.12%	5.84%	15.23%	10.0%	24.3%	78.8%
APM1(I164T)	0.008	NA	3.5 (0.25)	7.35 (0.07)	2.2×10^{-25}	2.62%	5.95%	15.51%	127.5%	24.6%	79.5%
APM1(H241P)	0.006	NA	8.44 (0.71)	7.25 (0.07)	0.062	0.09%	5.89%	15.25%	26.1%	24.4%	78.8%

Age, sex, and BMI adjusted ($N = 3310$).
f, frequency; (a), additive; (p ad), per allele; (p sex), per sex.

Table 5. Association of Obesity-Related Phenotypes, Adiponectin, and the rs12051272 Genotypes in Suita (1 + 2)

	P-value (response variable = each parameter)				Partial correlation coefficient		
	rs12 (a v)	rs12 (a v) pl adipo adj	pl adipo	pl adipo, rs12 (a v) adj	betw rs12 (a v) and each	betw rs12 (a v) and pl adipo	betw pl adipo and each
			A. Adjustment of age and sex				
Fasting insulin[a]	0.84	4.05×10^{-6}	9.09×10^{-89}	7.04×10^{-94}	−0.088	−0.22	−0.38
Fasting glucose[a]	0.66	2.33×10^{-3}	1.17×10^{-32}	1.77×10^{-34}	−0.058	−0.21	−0.23
HOMA-IR[a]	0.77	8.62×10^{-7}	1.78×10^{-96}	5.00×10^{-102}	−0.094	−0.22	−0.39
Fasting triglyceride[a]	0.89	1.74×10^{-5}	2.92×10^{-81}	4.48×10^{-85}	−0.082	−0.22	−0.36
BMI	0.09	3.53×10^{-7}	4.78×10^{-69}	1.55×10^{-75}	−0.097	−0.22	−0.31
			B. Adjustment of age, sex, and BMI				
Fasting insulin[a]	0.40	0.015	6.73×10^{-43}	1.77×10^{-44}	−0.047	−0.22	−0.26
Fasting glucose[a]	0.98	0.036	7.12×10^{-18}	1.03×10^{-18}	−0.040	−0.22	−0.17
HOMA-IR[a]	0.46	0.0053	3.16×10^{-49}	4.07×10^{-51}	−0.053	−0.23	−0.28
Fasting triglyceride[a]	0.73	0.00074	7.01×10^{-56}	3.78×10^{-58}	−0.065	−0.23	−0.30

[a]Calculated by using logarithm. rs12:rs12051271. a v, additive variable; pl adipo, plasma adiponectin; adj, adjustment; betw, between.

each metabolic phenotype and the rs12051272 genotypes (additive model) was −0.094 ($P = 8.62 \times 10^{-7}$), −0.088 ($P = 4.05 \times 10^{-6}$), −0.058 ($P = 2.33 \times 10^{-3}$), and −0.082 ($P = 1.74 \times 10^{-5}$), respectively. The results were consistent if BMI was included as an explanatory variable (Table 5B). This means the SNP genotype has a statistically significant negative effect on those phenotypes independent of plasma adiponectin levels, with rs12051272-G allele increasing these metabolic risks, but it is cancelled by its positive effect through increasing adiponectin levels simultaneously. The mechanism how the SNP genotype has effects on those phenotypes independent of plasma adiponectin levels remains to be elucidated.

From the other point of view, when we analyzed the effect of plasma adiponectin concentration on those phenotypes, the association became even stronger when adjusted for rs12051272 genotypes (Table 5A, B). That suggests when we use the plasma adiponectin levels as a marker for risk of cardiovascular events or MetS in the clinical practice, as proposed by several researchers, the SNP genotypes of *CDH13* gene have to be considered. Based on our results that SNPs for *CDH13* gene were strongly associated with HMW adiponictin, we tried to adjust HMW adiponectin instead of plasma adiponectin, but we only observed similar results between adjusting HMW adiponectin and plasma adiponectin levels (data not shown).

During preparation of this manuscript, a genome-wide association study in Korean population as well as in Filipino women reported that rs3865188:A>T in *CDH13* was indeed associated with adiponectin levels [Jee et al., 2010; Wu et al., 2010] as shown here. Also, it showed that G variant at rs12444338:G>T in linkage disequilibrium with rs3865188 had an increased promoter activity of *CDH13* gene in vitro [Jee et al., 2010]. However, our results did show that rs12051272 SNP genotypes were more significantly associated with adiponectin levels than rs12444338 SNP genotypes (Table 1 and Supp. Table S4), while rs12444338 SNP genotypes were in linkage disequilibrium with rs4783244 and rs12051272 (Supp. Table S5).

Discussion

Adiponectin is an adipokine, secreted specifically and abundantly by adipose tissues. It has attracted much attention because of its antidiabetic and antiatherosclerotic effects by sensitizing the body to insulin. It has been shown that adiponectin inhibits hepatic glucose production, enhances glucose uptake in muscle, increases fatty acid oxidation in both liver and muscle, and augments energy expenditure by enhancing uncoupling of ATP generation in mitochondria in vitro. Numerous studies have shown that plasma adiponectin concentration correlates negatively with fasting plasma insulin levels and insulin resistance measures. Lower plasma levels of adiponectin have been shown to be associated with obesity, type 2 diabetes, coronary artery disease, hyperlipidemia, hypertension, and the MetS. Some investigators suggest that it can be considered as a biomarker or a diagnostic marker to predict vulnerability for MetS [Martin et al., 2005; Santaniemi et al., 2006], cardiovascular events [Pischon et al., 2004], and in-stent restenosis after acute myocardial infarction [Kitta et al., 2008; Moldoveanu et al., 2008].

Adiponectin exists in three major oligomeric forms; a LMW trimer, a middle-molecular weight (MMW) hexamer, and HMW 12- to 18-mer. A small amount of globular form, possibly resulting from proteolytic cleavage, has also been described. Recently, HMW adiponectin has been especially attracting attention because the level of HMW adiponectin was reported to be more significantly associated with parameters related to glucose metabolism than other forms. The level of HMW adiponectin or the ratio of HMW to total adiponectin was shown to be more relevant to the prediction of insulin resistance [Lara-Castro et al., 2006].

T-cadherin (*CDH13*), which is expressed in endothelium and smooth muscle, has been identified as a receptor specific for HMW adiponectin [Hug et al., 2004]. The amino acid motif of T-cadherin is well conserved in higher eukaryote compared to that of E-cadherin, suggesting of some biological significance. It lacks a cytoplasmic domain and is anchored to the surface membrane via glycosyl phosphatidyl inositol (GPI) moiety, it is speculated that T-cadherin may act as a co-receptor along with other signaling molecules, but its physiological roles are largely unknown. T-cadherin has been shown to be more strongly expressed in regenerative endothelial cells and vascular smooth muscle cells in the region of atherosclerosis than in those of the normal artery, and the level of its expression is known to be correlated with the progression of atherosclerosis, implicating that it is playing some role in atherosclerotic changes.

We showed a single SNP rs12051272, tagging a corresponding haplotype constituting of rs12051272 and rs4783244, was significantly associated with the plasma adiponectin levels. The association of the rs12051272 SNP genotypes and plasma adiponectin levels was consistent across all studies. The effect size measured in eta squared in Suita study population suggested that the two variants rs12051272 in *CDH13* gene and Ile164Thr of *ADIPOQ* explained 3.28% and 2.62% of the variation in plasma adiponectin levels, respectively. Interaction between rs12051272 in the *CDH13* gene and +517T>C (Ile164Thr) within *ADIPOQ* gene was not statistically significant, though the combined effects of these two SNPs on plasma adiponectin concentrations were shown to be additive (data not shown). Although the rs12051272 showed the smaller

beta-coefficient than Ile164Thr, the contribution in the population was greater because of the higher MAF.

Previously, a similar GWAS study adiponectin was reported [Richards et al., 2009]. They used the same SNP platform, Illumina HumanHap550, as ours but failed to identify any *cis*- or *trans*-SNP of *CDH13* affecting the plasma adiponectin levels. Illumina HumanHap550 assay contains a probe for rs4783244 but not that for rs12051272. The haplotype structure data obtained from Human HapMap project showed that the MAF of rs4783244 was similarly high in both Caucasians and Japanese (0.46 and 0.32), while that of rs12051272 was much lower in Caucasians (0.01) than in Japanese (0.25). Based on our study results and the above, we estimated the power of detection for the SNP rs12051272 in Japanese as 0.992, while we did that in Caucasian as only 0.00002. Our result suggests that the haplotype constituting T allele of rs12051272 (G/T) and T allele of rs4783244 (G/T), tagged by a single rs12051272 SNP, has an effect in decreasing plasma adiponectin levels. Illumina HumanHap550 does not contain the probe for rs12051272, along with the too low MAF of this TT haplotype to be detected of significant association in Caucasian population that contains mostly GG and TG haplotypes, were the main reasons why GWAS in Caucasian population failed to identify the significant association. The difference of MAF may also partly explain the difference in the baseline plasma adiponectin levels between Japanese and American. Kadowaki et al. showed that the American men had been shown to have higher levels of adiponectin than the Japanese men despite higher levels of obesity [Kadowaki et al., 2006]. Since the majority of American had adiponectin-increasing rs12051272-G alleles, the mean plasma adiponectin levels can be apparently higher accordingly, but this does not mean the Americans are more resistant to risk phenotypes because this allele has an independent effect on augmenting insulin resistance as shown by the partial correlation coefficients. The mean difference in plasma adiponectin levels between GG and TT groups in Japanese was around 2 μg/ml consistently in both sexes in three independent groups, so the sole genotypic difference in T-cadherin gene may not explain the 6 μg/ml difference between Japanese and Americans.

Compared with the report of the genome-wide association study in Korean population as well as in Filipino women, our results did show that rs12051272 SNP genotypes were more significantly associated with adiponectin levels and especially with the levels of HMW adiponectin than rs12444338 SNP genotypes.

Our results provide two novel insights relating T-cadherin. First, in analyzing the physiological effects of plasma adiponectin, the analysis has to be adjusted for the SNP genotypes that would affect the 28% of SD changes in plasma adiponectin level per allele.

Several researchers have proposed to use the plasma adiponectin levels as a marker for risk of cardiovascular events or MetS in the clinical practice. For that purpose, the effect of the SNP genotypes, with the mean difference of 1 μg/ml per allele, on the levels of adiponectin is too large to ignore, especially in Japanese. Second, T-cadherin should have some unknown effect independent of adiponectin levels, since the same allele had an opposite effect judging from the results of partial correlation coefficient. The effect of the rs12051272 SNP also has to be elucidated. Since the SNP is located in the first intron of *CDH13* gene and the surrounding nucleotide sequence did not match the known transcription factor binding site or miRNA-targeted sequence, it is likely that the rs12051272 is a mere marker SNP tagging the phenotype-affecting haplotype present in 30-kb haplotype block covering from the promoter region to the first intron of CDH13. The idea that phenotype-affecting haplotype tagged by rs12051272 SNP would affect the baseline level of T-cadherin in the tissues is attractive, since the increasing amount of T-cadherin may capture the free adiponectin molecules in the plasma resulting in lowering plasma HMW adiponectin levels, simultaneously increasing the adiponectin signals in the peripheral vessel walls resulting in augmenting the effect of adiponectin per cell. Therefore, we may have to take the CHD13 genotypes as well as ADIPOQ genotypes into account if the plasma adiponectin levels is used as a marker for a risk of cardiovascular events or diabetes, though the attempt to compare the level of T-cadherin in peripheral vessel walls with the genotype will give us further evidence for this result.

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Feasibility Evaluation of a Remote Monitoring System for Implantable Cardiac Devices in Japan

A Prospective Analysis

Kenji ANDO,[1] MD, Junjiroh KOYAMA,[2] MD, Yoshihisa ABE,[3] MD, Toshiaki SATO,[4] MD, Morio SHODA,[5] MD, Yoshimitsu SOGA,[1] MD, Masakiyo NOBUYOSHI,[1] MD, Toshihiro HONDA,[2] MD, Koichi NAKAO,[2] MD, Ken TERATA,[3] MD, Ken KADOWAKI,[3] MD, Akiko MAEDA,[4] RN, Satoshi OGAWA,[4] MD, Tetsuyuki MANAKA,[5] MD, Nobuhisa HAGIWARA,[5] MD, *and* Kentaro DOI,[6] PhD

SUMMARY

The number of implanted cardiac devices has been growing steadily over the last several years. Systems to monitor device data remotely have been introduced with the goal of reducing follow-up burden for both patients and physicians. Since the introduction of telemedicine depends greatly on the situations that are unique to each country, the acceptance of cardiac device remote monitoring in Japan was analyzed.

A total of 203 patients who had previously undergone cardiac device implantation were enrolled. The subjects were provided with a CareLink Monitor that performed interrogation and transmission of device data at home, and then the physicians reviewed the data via a website at one and 3 months after baseline visits. A total of 470 transmissions were made. Questionnaires were completed by subjects and physicians to evaluate acceptance, ease of use, and satisfaction with the system. More than 87% of the subjects felt the Monitor was easy to use and nearly all of the physicians were satisfied with the system. A majority of patients felt reassured by having their devices assessed from a remote location and preferred the decreased number of clinic visits that were possible when using the Monitor. The patients spent an average of 168.2 minutes per clinic visit, whereas follow-up time was reduced to 13.0 minutes by remote monitoring. Physician consultation time was reduced by 2.7 minutes.

The CareLink Network was well accepted by both the patients and physicians. Underlying issues did emerge, but once they are overcome, the system appears to have great potential to improve the quality of care given by healthcare providers. (Int Heart J 2011; 52: 39-43)

Key words: Device follow-up, Telemedicine, Feasibility study

The number of patients treated for cardiovascular diseases with implantable devices such as pacemakers and implantable cardioverter defibrillators (ICD) has been growing rapidly in recent years. Current devices are capable of continuous cardiac monitoring and storage of long-term trends in cardiac rhythms, episodes, and physical conditions. These data are often interrogated and assessed during in-office visits with the use of appropriate programmers. An ever growing demand for implantable device follow-up is stressing the capability of clinics to levels that are difficult to sustain. Therefore, a reduction of the burden by any means possible would have a significant positive impact on the quality of care provided by these physicians.

One solution may be the remote monitoring of implantable cardiac devices. The Medtronic CareLink Network (Medtronic, Inc., Minneapolis, MN, USA)[1)] utilizes an internet-based patient information management system. It is comprised of a patient monitor (Monitor), which interrogates and transmits device data via a standard analogue telephone connection, a secure server, in which the transmitted data are stored, and a clinician website, which offers the physician secure data access to patient data. Remote monitoring of ICD[1)] and cardiac resynchronization therapy with defibrillators (CRT-D)[2,3)] have been studied in the United States and Europe. Since the feasibility and acceptance of telemedicine depend heavily on the social, geographical, and economical situations of a country, in the present study we conducted assessments of the ease of use, satisfaction level, and acceptance of this system by both patients and physicians in Japan, and also discuss the impacts of the method on patient and physician time and burden.

METHODS

This study was a prospective, observational, nonrandomized clinical trial conducted at 5 centers in Japan. It was ap-

From the [1] Department of Cardiology, Kokura Memorial Hospital, Fukuoka, [2] Division of Cardiology, Cardiovascular Center, Saiseikai Kumamoto Hospital, Kumamoto, [3] Division of Cardiology, Akita Medical Center, Akita, [4] Cardiology Division, Keio University School of Medicine, [5] Department of Cardiology, Tokyo Women's Medical University, and [6] Cardiac Rhythm Disease Management, Medtronic Japan Co., Ltd., Tokyo, Japan.

Address for correspondence: Kentaro Doi, PhD, Cardiac Rhythm Disease Management, Medtronic Japan Co., Ltd., Comodio Shiodome 5F, 2-14-1 Higashi Shimbashi, Minato-ku, Tokyo 105-0021, Japan.

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proved by the Institutional Review Board or Medical Ethics Committee of each study site, and was carried out in accordance with the Ethical Guidelines for Clinical Studies (Ministry of Health, Labour and Welfare, July 30, 2003, amended on December 28, 2004).

Study population: The targeted number of subject enrollments per center was 35 to 50, with an expected minimum overall total of 200. Patients with previously implanted CareLink-compatible cardiac devices who had analogue telephone connections at home and were willing to sign the informed consent were enrolled. The study population was comprised of patients who had undergone implantation of a brady pacemaker, ICD, or CRT-D.

Subject follow-up: All subjects received conventional medical care in addition to guidance concerning the use of the Monitor for device interrogation and data transmission. The subjects had scheduled in-office study visits at baseline and at 6-months follow-up. They were also instructed to interrogate and transmit their device data using the Monitor at one and 3 months after the baseline visit. Subjects and physicians were asked to complete questionnaires with respect to satisfaction and ease of use of the CareLink system.

Successful data transmission: During the baseline visit, subjects were trained on how to setup the Monitor at home and perform data transmission. The Monitor was sent to their house directly from the manufacturer and the subjects were asked to complete initial setup on their own or with the assistance of caregivers. When a data transmission session was successfully completed without troubleshooting telephone calls, it was considered a "Successful Transmission". The success rates were calculated for 4 time points; scheduled transmissions at 1 month and 3 months after the baseline visit and unscheduled transmissions which were transmitted during the periods prior to (pre-one-month) or subsequent to (post-one-month) the first scheduled transmission at one month after the baseline visit.

Time and burden impact: Time and burden impact was compared between clinic visits and data transmissions for both the physicians and subjects. Clinic visit time was defined as the total time required to travel to and from the clinic, waiting to be seen at the clinic, and for the actual medical procedure. To assess the impact of remote monitoring on subjects and their caregivers, if applicable, questionnaires were administered to the subjects during baseline visits with main focuses on travel time, and amount of work time missed by the subjects and caregivers. The time required to perform scheduled and unscheduled data transmissions were also noted after each attempt.

Physicians were asked to document the time required to complete the follow-up visits for each subject. When transmitted data was received from the subjects, physicians evaluated the data via the internet and noted the time they spent to perform this procedure.

Statistical analysis: Categorical data are expressed as numbers and percentile. Continuous data are presented as the mean and standard deviation. Comparisons of continuous variables were made using Student's *t*-test or the Tukey-Kramer method. In order to compare multiple categorical data, Ryan's method was used. A P value less than 0.05 was considered significant. A control arm was not established due to the observational nature of the study.

Results

Patient population: The first enrollment occurred on February 5, 2008, and the final follow-up was completed on March 19, 2009. The total number of subjects enrolled was 203. Twenty-two subjects withdrew from the study due to death (7), a subject leaving a study center (1), request for discontinuation by a subject (3), telephone line incompatibility (7), and subject lost to follow-up (4). Patient demographics are shown in Table I. Sixty-seven percent of the population was male, and mean age at baseline was 67.5 ± 13.6 years. More than half of the enrolled subjects were implanted with pacemakers, while 43.4% and 3.5% had ICD or CRT-D implantations, respectively. The most prominent cardiovascular condition was myocardial infarction with a prevalence of 18.2%. Sixty-six (32.5%) subjects had a history of atrial fibrillation. Other major conditions found

Table I. Patient Demographics

	(n = 203)
Gender: Male	136 (67.0%)
Age: Mean ± SD	67.5 ± 13.6
Device category	
IPG	108 (53.2%)
ICD	88 (43.4%)
CRT-D	7 (3.5%)
Cardiovascular history	
Cardiomyopathy	46 (22.7%)
Coronary artery disease	64 (31.5%)
Valve dysfunction	13 (6.4%)
Arrhythmia history	
Sick sinus syndrome	67 (33.0%)
Atrial arrhythmia	76 (37.4%)
Ventricular arrhythmia	110 (54.2%)
Conduction anomaly	66 (32.5%)

Table II. Proportion of Successful Transmissions

	Number of transmissions	Number of successful transmissions	% Success	95% CI
Pre-one-month unscheduled transmission	29	20	69.0	49.2-84.7
One-month scheduled transmission	190	132	69.5	62.4-75.9
Three-month scheduled transmission	183	169	92.3	87.5-95.8
Post-one-month unscheduled transmission	68	64	94.1	85.6-98.4
Total	470	385	81.9	78.1-85.3

CI indicates confidence interval.

Table III. Subject Satisfaction, Ease of Use, and Perspective

	Transmission			
	Unscheduled (Pre-one-month)	1 Month	3 Months	Unscheduled (Post-one-month)
Clarity of Monitor User Manual				
Very clear	6 (25%)	45 (26%)	54 (32%)	16 (32%)
Clear	13 (54%)	101 (59%)	108 (64%)	32 (64%)
Unclear	4 (17%)	24 (14%)	5 (3%)	1 (2%)
Very unclear	1 (4%)	0 (0%)	1 (1%)	1 (2%)
Ease of Monitor Set up				
Very easy	6 (25%)	55 (32%)	72 (43%)	18 (37%)
Easy	13 (54%)	91 (53%)	85 (51%)	28 (57%)
Difficult	4 (17%)	25 (15%)	8 (5%)	2 (4%)
Very difficult	1 (4%)	0 (0%)	2 (1%)	1 (2%)
Ease of Antenna Positioning				
Very easy	13 (54%)	81 (48%)	84 (50%)	27 (54%)
Easy	10 (42%)	82 (48%)	79 (47%)	19 (38%)
Difficult	1 (4%)	7 (4%)	3 (2%)	3 (6%)
Very difficult	0 (0%)	0 (0%)	2 (1%)	1 (2%)
Time Required for Transmission				
Very brief	0 (0%)	17 (10%)	25 (15%)	7 (14%)
Brief	17 (71%)	102 (61%)	124 (74%)	32 (65%)
Long	7 (29%)	41 (24%)	17 (10%)	8 (16%)
Very long	0 (0%)	8 (5%)	2 (1%)	2 (4%)
Overall Ease of Use of the Monitor				
Very easy	4 (17%)	51 (30%)	57 (34%)	15 (30%)
Easy	17 (71%)	97 (57%)	105 (63%)	32 (64%)
Difficult	2 (8%)	23 (13%)	5 (3%)	2 (4%)
Very difficult	1 (4%)	0 (0%)	1 (1%)	1 (2%)

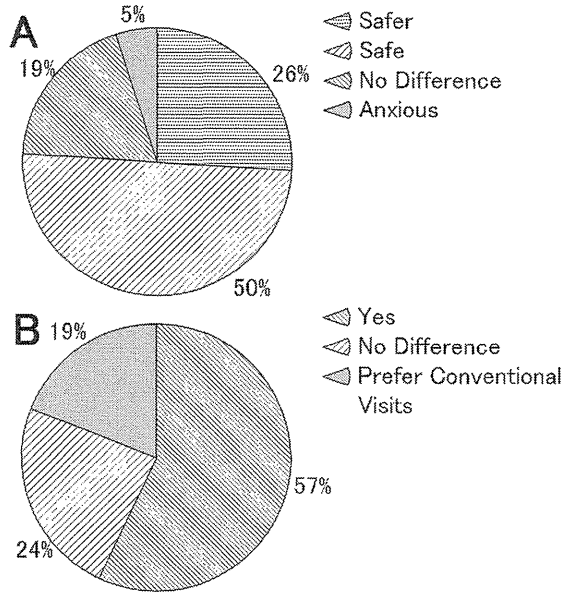

Figure. Patient perspective on the use of the remote monitoring system at the 6-month follow-up visit (n = 168). (**A**) How do you feel about physicians assessing the device data from a remote location? (**B**) Do you prefer the less frequent clinic visits when using the remote monitoring system?

among the population were hypertension in 83 (40.9%) and experience of cardiac syncope in 78 (38.4%).

Data transmission: Seven subjects discontinued the study due to telephone line incompatibility caused by their fiber-optic telephone systems and/or telephone service carriers. Internet-based telephone systems such as this fiber-optic network are gaining popularity and the number of analogue telephone lines is constantly decreasing in Japan, so this issue must be addressed in a timely manner. The success rate for data transmission using the Monitor is shown in Table II. A total of 470 transmissions were attempted during the study, of which 385 (81.9%) were classified as successful. Sixty-nine percent success was achieved for pre-one-month unscheduled and 1-month scheduled transmissions, whereas subsequent transmissions had a much higher success rate of over 92%. Multiple comparisons of each time point found that the differences in the success rates between pre-one-month unscheduled versus 1-month scheduled transmissions, and between 3-month scheduled versus post-one-month unscheduled transmissions were not significant. The difference in success rate between the first-time transmissions and those after was statistically significant ($P <$ 0.004). There were 97 instances of troubleshooting calls. The most frequent (33%) reason for a troubleshooting call was an incorrect configuration between the pulse and tone dialing. The Monitor is capable of handling either a pulse or tone dialing method when placing a phone call. The setting could be altered by flipping a switch on the device. The dialing method must match to what is being offered by the telephone service carriers, otherwise the device will not be able to place a call before initiating an internet connection. Communication environment related difficulties were seen in 20% and inquiries regarding the usage of the Monitor were posted from 21% of subjects. Twenty-four (25.1%) calls were considered to be irrelevant inquiries.

Subject satisfaction and ease of use: Feedback from the subjects on the use of the Monitor is summarized in Table III. A majority of the subjects found the user manual to be clearly written and understandable. Setting up the Monitor and positioning of the antenna on their implanted devices for interrogation was considered easy or very easy by more than 79% of subjects. Overall ease of use was also favorably perceived.

Even though a large proportion of subjects provided positive feedback, somewhat mixed results were seen with the time required for transmission. This was considered to be caused by the subjective nature of the inquiry. One person may feel 10 minutes is very long, while another may feel it is short.

The results indicated that the subjects felt more confident with the Monitor after using it the first time since shorter transmission times and increases in affirmative feedback were seen with later transmissions. This suggests the importance of training at the clinic before initiating the use of this remote monitoring system.

Do you like remote monitoring?: During their final study visit, ie, 6-month follow-up visit, the subjects were asked to evaluate their experience with remote monitoring. A majority (76%) of the subjects felt it was safe or safer than before to have their device data monitored remotely by the physicians (Figure). In contrast, 5% felt some anxiety. Certain patients may need to have all consultations in person with their physician. An appropriate method for selection of suitable patients will be needed when remote monitoring becomes the standard of care.

More than half of the subjects replied that they preferred the lower number of clinic visits associated with the use of a remote monitoring system.

Physician satisfaction and ease of use: Feedback from the physicians regarding the quality of the website is summarized in Table IV. All the responses were positive, with the exception of a single doctor for the one-month transmission. Physicians were confident about the accessibility and search capability of the secure website. Work flow applied to unscheduled transmissions at individual and organizational levels has yet to be standardized, and it may have had some influence on the somewhat lower ratings seen for unscheduled transmissions. The device data collected through the website were comparable to the data obtained with direct device interrogation at the clinic, and they were deemed satisfactory to all physicians, except on one occasion.

Comparison of time burden impact: The time required for the baseline clinic visits and each data transmission for the subjects are compared in Table V. Direct comparison of the actual time required for data transmission and clinic visits revealed that the time necessary for data transmission was significantly shorter than for a clinic visit.

A majority of the subjects (54%) used cars as the mode of transportation to the clinic. Assistance by a caregiver was needed by 58% and 45% of the study population when going to their clinic visits at baseline and 6-month follow-up, respectively. When making these visits, more than one quarter of the subjects and one third of the persons accompanying the subject missed work that day (data not shown).

Physicians spent a mean of 9.05 ± 4.84 minutes and 6.35 ± 5.10 minutes for scheduled in-clinic device follow-ups and review of transmitted data, respectively. The difference of 2.70 minutes was statistically significant ($P < 0.001$). Further comparison of individual time points revealed that unscheduled in-clinic visits took a significantly longer period of time, whereas the difference between unscheduled transmissions and regular in-clinic visits was not significant.

Table IV. Physician Satisfaction and Ease of Use

	Transmission		
	1 Month	3 Months	Unscheduled
Ease of Access to the Website			
Very Easy	122 (70%)	126 (70%)	37 (38%)
Easy	51 (29%)	54 (30%)	60 (62%)
Difficult	1 (1%)	0 (0%)	0 (0%)
Very Difficult	0 (0%)	0 (0%)	0 (0%)
Ease of Search through the Website			
Very Easy	113 (65%)	117 (65%)	32 (33%)
Easy	60 (34%)	63 (35%)	65 (67%)
Difficult	1 (1%)	0 (0%)	0 (0%)
Very Difficult	0 (0%)	0 (0%)	0 (0%)
Data Comparability against Conventional Follow-up			
Strongly Agree	71 (41%)	50 (28%)	9 (9%)
Agree	102 (59%)	130 (72%)	88 (91%)
Disagree	1 (1%)	0 (0%)	0 (0%)
Strongly Disagree	0 (0%)	0 (0%)	0 (0%)

Discussion

It is well accepted that telemedicine may have the potential to bring about major improvements in healthcare systems, but in reality, it is reported that 75% of telemedicine initiatives failed during the operational phase.[4] Tanriverdi and Iacono (1999) introduced a theory stating that knowledge barriers inhibit the diffusion of telemedicine. Those barriers were based on technical, behavioral, economical, and organizational aspects.[5] Broens, *et al* (2007) further expanded this theory by adding a policy and legislation standpoint.[6] All of these barriers must be lowered in order to achieve successful deployment and long-term implementation of remote monitoring systems in daily medical practice.

In the present study, technical difficulties unique to Japan, such as telephone line issues, were encountered. Some of these concerns were solved during the course of the study, but since 30% of the subjects had difficulty with their first transmissions, improvements in training and support are needed. From a behavioral point of view, it is clear that the CareLink Network was well accepted by both patients and physicians. To achieve long-term success with the system, its benefits must be promoted based on the medical evidence obtained thus far.

It is evident that patients are sure to benefit from the time, cost, and labor savings. However, for physicians and clinics, only a slight reduction in follow-up time was demonstrated. This study may have been a success because it was funded research and the follow-up duration was short. Further discussion is necessary to assess the correlation between the reduction of labor and economical impact for the physicians. Each organization must set up their own standard operation procedure in order to run the system smoothly. Current forms of legislation and policy are not best suited for new telemedicine technologies. If these could be addressed and the procedures

Table V. Time Burden Impact

	Clinic Visit			Transmission		
(Minutes)	Baseline	6 Months	Unscheduled	1 Month	3 Months	Unscheduled
Subject	(n = 196)	-	-	(n = 169)	(n = 166)	(n = 74)
Mean	168.2 ± 95.7	-	-	14.7 ± 23.7	9.7 ± 5.6	16.3 ± 26.2
Median	150	-	-	10.0	10.0	10.0
Min-Max	10.0-510.0	-	-	2.0-270.0	3.0-40.0	1.0-180.0
Physician	(n = 153)	(n = 130)	(n = 13)	(n = 173)	(n = 180)	(n = 95)
Mean ± SD	9.4 ± 5.2	8.7 ± 4.3	14.8 ± 6.9	6.6 ± 4.7	6.2 ± 5.5	7.9 ± 6.1
Median	8.0	7.0	15.0	5.0	5.0	9.0
Min-Max	4.0-20.0	5.0-20.0	5.0-30.0	1.0-20.0	1.0-30.0	1.0-30.0

standardized, the feasibility of such systems would greatly improve.

The pros and cons of telemedicine are currently being discussed.[7,8] Without question, a face-to-face consultation with a physician can never be totally replaced by telemedicine. However, the dramatic increase in the elderly population will have a great impact on device clinics due to the ever-growing demand for cardiac devices. Actions must be taken in order to decrease the burden on such physicians to enhance the overall quality of care. Reducing follow-up time by the use of remote monitoring may be a promising solution.

Medical economic evaluation using the CareLink Network has been performed in Europe.[9] Although the results can not be directly applied to the situation in Japan, it is possible to perhaps predict the potential influence the system may have. The objective of the current study was not to evaluate symptom oriented data transmission from the patients. In order to collect more evidence regarding the clinical efficiency of the CareLink Network, randomized trials to evaluate disease management and patient outcomes as well as medical economical endpoints are needed.

Limitations: The design of the present study was nonrandomized and uncontrolled. Some of the data collected was subjective in nature, and some data were missing since a proportion of the questionnaires could not be retrieved from the subjects and physicians.

Conclusions: The present study has demonstrated that remote monitoring of implantable cardiac devices utilizing the CareLink Network System was well accepted by both a representative population of patients and physicians in Japan. Such a system has the potential to improve clinical efficiency and the way cardiac disease is managed. Certainly there are hurdles that must be cleared before widespread employment of the system, but once these obstacles are overcome, the system will most likely have an enormous impact on how care is delivered to patients. Physicians may also benefit from the reduction in time needed for follow-up.

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ORIGINAL ARTICLE

Accelerated BMIPP uptake immediately after reperfused ischemia in the isolated rat heart model

Kenji Fukushima · Mitsuru Momose · Chisato Kondo · Nobuhisa Hagiwara · Shuji Sakai

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Abstract
Objective ^{123}I-beta-methyl iodophenyl pentadecanoic acid (BMIPP) can visualize myocardial fatty acid metabolism and has extensive potential for diagnosing cardiac diseases such as acute coronary syndrome in the clinical setting. Increased BMIPP uptake with decreased perfusion occasionally occurs under acute reperfusion ischemia and the kinetics of BMIPP remain unclear. The present study uses the isolated rat heart model to measure kinetic changes in BMIPP under acute reperfusion ischemia.
Methods Male Wistar rats were allotted to normal control (NG), mild (MG) and severe (SG) ischemia groups. The hearts were perfused according to the Langendorff method at a constant flow rate, and BMIPP wash-in and wash-out were studied. No-flow ischemia was applied for 15 and 30 min to the MG and SG groups, followed immediately by the wash-in and wash-out study. Whole heart radioactivity was determined using an external gamma detector throughout the experiment. Rates of myocardial uptake (K_1, mL/min) and clearance (k_2, min^{-1}) were generated using a compartmental model analysis. The same procedures and protocols were performed using ^{99m}Tc-sestamibi (MIBI) as a perfusion study.
Results Perfusion pressure significantly increased and mean heart rate significantly decreased in the severe ischemia group (heart rate: 244 ± 76, 304 ± 105 and 94 ± 140 bpm; perfusion pressure: 67 ± 13, 101 ± 31 and 160 ± 84 mmHg for NG, MG and SG, respectively). MIBI-K_1 significantly decreased, whereas BMIPP-K_1 increased in the MG and SG groups (MIBI-K_1: 3.45 ± 1.10, 1.95 ± 0.82, and 1.05 ± 0.13 mL/min; BMIPP-K_1: 3.06 ± 0.88, 3.91 ± 0.87, and 4.94 ± 1.51 mL/min for NG, MG and SG, respectively) with an inverse relationship to the severity of ischemia. MIBI-k_2 increased markedly in severe ischemia (NG vs. MG: $p < 0.05$), whereas BMIPP-k_2 did not change in the ischemic groups (MIBI-k_2: 0.00072 ± 0.0011, 0.00038 ± 0.00076 and 0.043 ± 0.033; BMIPP-k_2: 0.0056 ± 0.0028, 0.0029 ± 0.0010 and 0.0037 ± 0.0022 min^{-1} for NG, MG and SG, respectively).
Conclusion Myocardial BMIPP uptake increased immediately upon reperfusion after no-flow ischemia, and was inversely related to the severity of ischemia. The increased uptake was not due to reduced clearance, but to accelerated extraction.

Keywords Fatty acid metabolism · I-123 BMIPP · Isolated rat heart · Myocardial ischemia · SPECT

Introduction

Myocardial fatty acid metabolism constitutes the major portion of the energy pathway that supplies the ATP required for healthy cardiac contraction. Free fatty acids account for 50–70% of the major energy source under normal conditions [1, 2]. Because ATP production via fatty acid metabolism requires abundant oxygen, metabolism can shift to glucose when the myocardium is compromised by ischemic or hypoxic insult. Therefore, to detect dysfunctional fatty acid metabolism is considered to be quite important.

K. Fukushima · M. Momose (✉) · C. Kondo · S. Sakai
Department of Diagnostic Imaging and Nuclear Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan
e-mail: mmomose@rad.twmu.ac.jp

N. Hagiwara
Department of Cardiology, Tokyo Women's Medical University, Tokyo, Japan