ORIGINAL ARTICLE

Effect of obesity on the association between *MYL2* (rs3782889) and high-density lipoprotein cholesterol among Korean men

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High-density lipoprotein (HDL) cholesterol levels are associated with a decreased risk of coronary artery disease. Several genome-wide association studies that have examined HDL cholesterol levels have implicated myosin light chain 2 regulatory cardiac slow (*MYL2*) as a possible causal factor. Herein, the association between the rs3782889 single-nucleotide polymorphism (SNP) in the *MYL2* gene and HDL cholesterol levels was tested in the Korean population. A total of 4294 individuals were included in a replication study with *MYL2* SNP rs3782889. SNP rs3782889 in the *MYL2* gene was associated with mean HDL cholesterol level (effect per allele, -1.055 mg dl^{-1}, $P = 0.0005$). Subjects with the CT/CC genotype had a 1.43-fold (range 1.19–1.73-fold) higher risk of an abnormal HDL cholesterol level (<40 mg dl^{-1}) than subjects with the TT genotype. When analyzed by sex, the *MYL2* association was stronger in men than that in women. When analyzed by body mass index (BMI), the *MYL2* association was much stronger in male subjects with BMI $\geqslant 26.44$ kg m^{-2} (odds ratio (OR) = 2.68; 95% confidence interval (CI) = 1.87–3.84; $P < 0.0001$) than that in male subjects with BMI <26.44 kg m^{-2}. When compared with subjects having the TT genotype and BMI <26.44 kg m^{-2}, ORs (95% CI) were 3.30 (2.41–4.50) in subjects having the CT/CC genotype and BMI $\geqslant 26.44$ kg m^{-2} (P for interaction <0.0001). Our results clearly demonstrate that genetic variants in *MYL2* influence HDL cholesterol levels in Korean obese male subjects.

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INTRODUCTION

High-density lipoprotein cholesterol (HDL-C) levels are associated with decreased risk of cardiovascular disease (CVD).[1–3] Several genome-wide association studies (GWASs) that have examined HDL-C levels reported that the myosin light chain 2 regulatory cardiac slow (*MYL2*; MIM 160781) gene is a candidate causal gene.[4,5] The *MYL2* gene has also been linked with multiple CVDs.[6–8] Continental Asian populations tend to have lower levels of serum cholesterol than those of Europeans.[9,10] Recent GWASs in Asian populations have reported that *MYL2* single-nucleotide polymorphisms (SNPs) are strongly associated with HDL-C levels.[5] Another recent GWAS on coronary artery disease reported that the rs3782889 SNP in *MYL2* is strongly associated with HDL-C level.[8] However, they did not analyze the association between rs3782889 and HDL-C in subjects with other risk factors, such as smoking status and body mass index (BMI).

MYL2 regulates myosin ATPase activity in smooth muscle.[11,12] In this study, the association between HDL-C level and the rs3782889 *MYL2* SNP was analyzed in a sample of Korean volunteers. The association was also analyzed considering BMI and smoking status.

MATERIALS AND METHODS

Study population

A total of 4294 subjects were the participants who had routine health examinations in the Health Promotion Center in University Hospitals from 1994 to 2012. Among 4294 subjects, 1810 subjects were CVD cases identified by the health insurance reimbursement data from the NHIC. CVD was defined according to the codes of the International classification of Disease (ICD), 10th Revision (I00-I99). In total, 137 subjects who were undergoing lipid-lowering therapy were excluded, and other subjects were excluded owing to missing BMI and HDL-C level data. Thus, the final population included 4025 subjects. Among 4025 subjects, 2403 subjects were the healthy subjects. Other 1622 subjects were CVD patients. The Institutional Review Board of Human Research of Yonsei University approved the protocols for this study, and written informed consent was obtained from all the subjects before enrollment.

Data collection

Each participant was interviewed using a structured questionnaire to collect their personal history of cigarette smoking (never smoked, ex-smoker or current smoker) and demographic characteristics (age, sex and so on). Waist circumference was measured midway between the lower rib and iliac crest. Light clothing was worn to measure weight and height.

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BMI was calculated as the subject's weight (kg) divided by the square of the subject's height (m^2).

Serum was separated from peripheral venous blood samples obtained from each participant after a 12-h fast and stored at $-70\,^{\circ}C$ until clinical chemistry assays. Biomarkers of metabolic syndrome, including fasting blood glucose, total cholesterol, triglycerides and HDL-C, were measured with a Hitachi-7600 analyzer (Hitachi, Tokyo, Japan). Quality control was conducted in accordance with the procedures recommended by the Korean Association of Laboratory Quality Control.

Table 1 General characteristics of the study population

Subjects	All	Men	Women
N	4025	2744	1281
	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
Age, year	51.9 ± 10.2	51.7 ± 10.2	52.3 ± 10.1
Waist circumference, cm	84.0 ± 9.0	87.1 ± 7.7	78.5 ± 8.7
Body mass index, kg m^{-2}	24.4 ± 2.9	24.8 ± 2.8	23.4 ± 3.1
Fasting blood sugar, mg dl^{-1}	96.8 ± 22.7	98.7 ± 24.1	92.8 ± 18.9
Systolic blood pressure, mmHg	121.8 ± 14.5	123.3 ± 13.9	118.6 ± 15.2
Diastolic blood pressure, mmHg	78.3 ± 10.8	79.9 ± 10.6	74.9 ± 10.6
HDL cholesterol, mg dl^{-1}	50.9 ± 11.6	48.2 ± 10.1	56.7 ± 12.4
LDL cholesterol, mg dl^{-1}	117.2 ± 31.6	117.1 ± 31.6	117.2 ± 31.7
Triglyceride, mg dl^{-1}	143.6 ± 97.4	157.3 ± 101.8	114.3 ± 79.8
Gamma glutamyl transferase, IU l^{-1}	40.5 ± 51.5	49.1 ± 58.6	22.1 ± 21.3
	%	%	%
Smoking status			
Ex	27.8	39.2	2.3
Current	27.5	38.2	3.7
Cardiovascular disease	40.3	42.9	34.7
Abnormal HDL cholesterol (<40 mg dl^{-1})[a]	14.5	18.5	30.9

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]An abnormal high-density lipoprotein cholesterol (HDL-C) level in women was <50 mg dl^{-1}.

The rs3782889 *MYL2* gene SNP was genotyped via the TaqMan reaction.[13] Duplicate genotyping for 1–2.5% of all samples was carried out as a quality control measure. Only those SNPs with a concordance rate >99% in duplicates and a genotype success rate >98% were included in subsequent association analyses.

Statistical analysis

Data are expressed as means ± s.d. Most statistical analyses were performed using PLINK and SAS ver. 9.2 (SAS Institute, Cary, NC, USA). Each SNP was tested for possible effects on HDL-C level under an additive model. The multivariate linear regression models used in the study incorporated covariates (age, sex and BMI). Multiple logistic regression analysis was also performed. BMI and waist circumference were divided by quartiles. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to examine the association between the *MYL2* SNP and abnormal HDL-C levels (<40 mg dl^{-1} for men and <50 mg dl^{-1} for women; NCEP ATP III). All statistical tests were two-sided, and $P < 0.05$ was considered significant.

RESULTS

Mean age was 51.7 for male subjects and 52.3 for female subjects (Table 1). This sample of Korean volunteers had a low mean HDL-C level. Mean HDL-C level was lower in males (48.2 mg dl^{-1}) than that in females (56.7 mg dl^{-1}). About 14.5% of the subjects had abnormal HDL-C levels (<40 mg dl^{-1}). Of the sample data set, 38.2% of men and 3.7% of women were current smokers. And 42.9% of men and 34.7% of women were CVD patients.

Table 2 shows the *P*-values from a linear regression model for HDL-C levels in the cohort sample when age, sex and BMI were included as covariates. The rs3782889 SNP in the *MYL2* gene was associated with mean HDL-C level (effect per allele, -1.055 mg dl^{-1}, $P = 0.0005$). When analyzed in healthy subjects ($N = 2403$), the association was stronger (effect per allele, -1.472 mg dl^{-1}, $P = 0.0003$). The rs3782889 SNP in the *MYL2* gene was also associated with mean GGT level (effect per allele, -5.525 mg dl^{-1}, $P < 0.0001$).

The association between the *MYL2* gene SNP rs3782889 and abnormal HDL-C level was also examined (Table 3). Subjects with

Table 2 Association between the rs3782889 single-nucleotide polymorphism in the *MYL2* gene and lipid levels based on a linear regression model

	Genotypes				
	TT	CT	CC		
Phenotypes	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Effect(mg dl^{-1})	P-value
All subjects	(N = 2669)	(N = 1218)	(N = 138)		
HDL cholesterol, mg dl^{-1}	51.3 ± 11.6	50.1 ± 11.5	49.5 ± 11.6	−1.055	0.0005
LDL cholesterol, mg dl^{-1}	116.5 ± 31.0	118.6 ± 33.1	117.8 ± 31.0	1.802	0.0566
Triglyceride, mg dl^{-1}	143.7 ± 87.1	143.6 ± 118.2	141.1 ± 84.1	−0.216	0.9354
Body mass index, kg m^{-2}	24.4 ± 3.0	24.3 ± 2.9	24.3 ± 2.9	−0.158	0.0560
Waist circumference, cm	84.2 ± 9.1	83.8 ± 8.9	83.4 ± 8.5	−0.258	0.0726
Gamma glutamyl transferase, IU l^{-1}	43.3 ± 56.4	35.2 ± 39.6	33.5 ± 37.8	−7.222	<0.0001
Healthy subjects	(N = 1592)	(N = 721)	(N = 90)		
HDL cholesterol, mg dl^{-1}	52.5 ± 12.4	50.8 ± 12.1	50.3 ± 12.5	−1.472	0.0003
LDL cholesterol, mg dl^{-1}	118.8 ± 29.4	120.8 ± 31.5	122.8 ± 28.3	2.183	0.0445
Triglyceride, mg dl^{-1}	135.5 ± 80.5	133.7 ± 90.2	129.5 ± 62.6	−1.761	0.5322
Body mass index, kg m^{-2}	24.1 ± 2.9	23.9 ± 2.9	24.0 ± 2.7	−0.258	0.0117
Waist circumference, cm	82.3 ± 9.1	81.6 ± 9.0	81.6 ± 8.3	−0.342	0.0676
Gamma glutamyl transferase, IU l^{-1}	38.0 ± 39.3	32.1 ± 28.0	27.5 ± 24.1	−5.525	<0.0001

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; *MYL2*, myosin light chain 2.
Estimated effect size (β) and *P*-value in the multiple linear regression model considered age, sex and body mass index in the additive model.

the CT/CC genotype had a 1.43-fold (range, 1.19–1.73-fold) higher risk of an abnormal HDL-C level (<40 mg dl^{-1}) than those with the TT genotype. When analyzed by sex, the association between *MYL2* and abnormal HDL-C level was stronger in men than that in women.

The analysis by quartiles of BMI in male subjects was presented in Table 4. The association between *MYL2* and HDL-C level was much stronger in male subjects with BMI $\geqslant 26.44$ kg m^{-2} (OR = 2.68; 95% CI = 1.87–3.84, $P<0.0001$) than that in male subjects with BMI <26.44 kg m^{-2}. In the analysis by quartiles of waist circumference in male subjects, the association between *MYL2* and HDL-C level was much stronger in male subjects with WC $\geqslant 92$ cm (OR = 2.36; 95% CI = 1.57–3.56, $P<0.0001$) than that in male subjects with WC <92 cm. The association between *MYL2* and HDL-C level was stronger in current smokers ($P=0.0362$) and ex-smokers ($P=0.0159$) than that in nonsmokers ($P=0.7304$).

Table 5 indicates age-adjusted ORs for abnormal HDL cholesterol levels according to *MYL2* (rs3782889) genotype in strata of BMI, waist circumference and smoking status among Korean men. When compared with subjects having the TT genotype and BMI <26.44 kg m^{-2}, ORs (95% CI) were 3.30 (2.41–4.50) in subjects having the CT/CC genotype and BMI $\geqslant 26.44$ kg m^{-2} (P for interaction <0.0001). With regard to waist circumference, ORs (95% CI) were 2.89 (2.02–4.14) for subjects with the CT/CC genotype and WC $\geqslant 92$ cm compared with subjects with the TT genotype and WC <92 cm (P for interaction = 0.0060).

DISCUSSION

In a cohort of 4025 subjects, the rs3782889 SNP in the *MYL2* gene was associated with decreased HDL-C level, consistent with previous studies. In a recent GWAS of coronary artery disease, the rs3782889 SNP was strongly associated ($P=3.95\times10^{-14}$).[8] Another SNP in the 12q24 region, rs12229654 near the *MYL2* gene, is associated with HDL-C ($P=3.41\times10^{-23}$), hypertension ($P=1.79\times10^{-8}$), levels of gamma glutamyl transpeptidase ($P=8.76\times10^{-58}$) and plasma glucose levels in KARE study subjects.[4,5,14] However, a more moderate association with the SNP was observed herein. One possible reason for the difference in the result is that mean HDL-C levels in the present study were much higher than those in the KARE subjects. In recent GWAS, the *MYL2* gene was also associated with type 1 diabetes mellitus and clinically defined gout.[15,16]

In the present study, we found that *MYL2* SNP had a stronger association with HDL-C cholesterol levels in men than that in women, which is similar to several previous studies on the *MYL2* gene. A recent GWAS study reported a male-specific association between the rs3782889 SNP in the *MYL2* gene and hypertension.[4] In another

Table 3 Odds ratios (ORs) of the polymorphic rs3782889 *MYL2* genotypes for HDL-cholesterol levels[a] in the population ($n=4025$)

Subjects	Genotype	Normal ($\geqslant 40$ mg dl^{-1}) N (%)	Abnormal (<40 mg dl^{-1})[b] N (%)	OR (95% CI)	P-value
All	TT	2321 (67.5)	348 (59.5)	1.00 (reference)	
	CT /CC	1119 (32.5)	237 (40.5)	1.43 (1.19–1.73)	0.0001
Men	TT	1496 (66.9)	297 (58.6)	1.00 (reference)	
	CT/ CC	741 (33.1)	210 (41.4)	1.49 (1.22–1.82)	0.0001
Women	TT	608 (68.7)	268 (67.7)	1.00 (reference)	
	CT/ CC	277 (31.3)	128 (31.6)	1.08 (0.83–1.40)	0.5997

Abbreviations: CI, confidence interval; *MYL2*, myosin light chain 2.
[a]Adjusted for age, sex and body mass index.
[b]An abnormal high-density lipoprotein cholesterol (HDL-C) level in women was <50 mg dl^{-1}.

Table 4 Odds ratios (OR) of polymorphic rs3782889 *MYL2* genotypes for HDL-cholesterol levels[a] in Korean men ($n=2744$)

Subjects	Genotype	Normal ($\geqslant 40$ mg dl^{-1}) N (%)	Abnormal (<40 mg dl^{-1}) N (%)	OR (95% CI)	P-value
BMI <23.05	TT	400 (65.7)	44 (56.4)	1.00 (reference)	
	CT/CC	209 (34.3)	34 (43.6)	1.48 (0.92–2.39)	0.1080
$23.05<$ BMI $\leqslant 24.73$	TT	361 (62.5)	73 (68.2)	1.00 (reference)	
	CT/CC	217 (37.5)	34 (31.8)	0.77 (0.49–1.19)	0.2414
$24.73<$ BMI $\leqslant 26.44$	TT	352 (66.0)	93 (60.8)	1.00 (reference)	
	CT/CC	181 (34.0)	60 (39.2)	1.28 (0.88–1.86)	0.1959
BMI $\geqslant 26.44$	TT	383 (74.1)	87 (51.5)	1.00 (reference)	
	CT/CC	134 (25.9)	82 (48.5)	2.68 (1.87–3.84)	<0.0001
WC <82	TT	270 (63.4)	28 (62.2)	1.00 (reference)	
	CT/CC	156 (36.6)	17 (37.8)	1.02 (0.56–1.98)	0.8749
$82<$ WC $\leqslant 87$	TT	276 (64.6)	47 (58.8)	1.00 (reference)	
	CT/CC	151 (35.4)	33 (41.3)	1.31 (0.80–2.13)	0.2840
$87<$ WC $\leqslant 92$	TT	285 (68.4)	73 (64.0)	1.00 (reference)	
	CT/CC	132 (31.7)	41 (36.0)	1.21 (0.78–1.87)	0.3878
WC $\geqslant 92$	TT	300 (72.3)	66 (52.4)	1.00 (reference)	
	CT/CC	115 (27.7)	60 (47.6)	2.36 (1.57–3.56)	<0.0001
Nonsmokers	TT	334 (67.9)	158 (32.1)	1.00 (reference)	
	CT/CC	64 (66.0)	33 (34.0)	1.09 (0.68–1.72)	0.7304
Ex-smokers	TT	551 (65.7)	288 (34.3)	1.00 (reference)	
	CT/CC	103 (56.3)	80 (43.7)	1.49 (1.08–2.07)	0.0159
Current smokers	TT	515 (65.5)	271 (34.5)	1.00 (reference)	
	CT/CC	119 (57.2)	89 (42.8)	1.40 (1.02–1.91)	0.0362

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; WC, waist circumference.
[a]Adjusted for age.

Table 5 Age-adjusted odds ratios (ORs) for abnormal HDL-cholesterol levels[a] according to *MYL2* (rs3782889) genotypes in strata of BMI, waist circumference and smoking status in Korean men ($n = 2744$)

		OR (95% CI)		
Subjects	No. of subjects by genotypes	TT	CT/CC	P for interaction
Body mass index				<0.0001
<26.44 kg m^{-2}	1323/735	1.00 (reference)	1.12 (0.88–1.43)	
⩾26.44 kg m^{-2}	470/216	1.23 (0.94–1.63)	3.30 (2.41–4.50)	
Waist circumference				0.0060
<92 cm	979/530	1.00 (reference)	1.17 (0.88–1.55)	
⩾92 cm	366/175	1.23 (0.89–1.69)	2.89 (2.02–4.14)	
Smoking status				0.2694
Never smokers	398/191	1.00 (reference)	1.09 (0.69–1.72)	
Ever smokers	1288/728	1.10 (0.81–1.50)	1.60 (1.16–2.20)	

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; *MYL2*, myosin light chain 2.
[a]Adjusted for age.

recent study, the association between BMI and *MYL2* gene SNP was significantly stronger among men than among women.[17]

Several studies have reported that HDL-C level is regulated by environmental or lifestyle risk factors, such as obesity and smoking.[18,19] A recent GWAS identified a novel BMI-associated locus near *MYL2* (rs12229654) gene.[17] In the present study, the rs3782889 SNP in the *MYL2* gene was still associated with mean HDL-C level even when adjusted by BMI. We also found that the association between *MYL2* (rs3782889) and HDL cholesterol levels can be modified by obesity among Korean men. However, the underlying mechanism has been still unknown. In a study of young type 2 Zucker diabetic fatty rats, some cardiac muscle proteins (*Myh6* and *Myl2*) were downregulated in Zucker diabetic fatty heart compared with control heart.[20] In a cohort of *MYL2* p.(E22K) founder mutation carriers, the presence of an additional risk factor for hypertrophy such as hypertension and obesity increased the disease penetrance of hypertrophic cardiomyopathy ($P = 0.0005$; combined OR $= 39.47$).[21] In the present study, ORs (95% CI) for CVDs were 3.30 (2.41–4.50, $P = 0.0027$) in subjects having the CT/CC genotype and BMI ⩾26.44 kg m^{-2} compared with subjects having the TT genotype and BMI <26.44 kg m^{-2} (data not shown). We also examined the association between the *MYL2* SNP and HDL-C by smoking status. The association was slightly stronger in current smokers than in nonsmokers. To our knowledge, there are no previous studies that evaluated modification of the association of *MYL2* and HDL cholesterol by lifestyle risk factors, such as obesity and smoking, among Korean men.

MYL2 encodes the myosin light chain and is involved in heart morphogenesis. Downregulation of *MYL2* may have a role in coronary artery disease.[11,12,22,23] *MYL2* also mediates the AMP-activated protein kinase pathway, which regulates energy homeostasis in eukaryotes.[24] The SNP rs3782889 is located in intron 1 of the *MYL2* gene. Differences in the frequency of rs3782889 have been reported in different populations. The C allele frequency is 7.2% in Europeans, and is lower in sub-Saharan Africans (5.3%) and Mexican Americans (1.0%). The frequency in East Asians was 20.9% in Han Chinese in Beijing and 19.2% in Japanese according to HapMap data (NCBI website). In the present study, we found a C allele frequency of 18.5%.

Genetic studies of lipid levels in Asian populations may not necessarily identify the same set of susceptibility genes as those in European-derived populations. However, this Korean cohort showed that the *MYL2* gene on chromosome 12 is associated with serum HDL-C levels in Korean men. The association was much stronger in male obese subjects and smokers than that in leaner nonsmoking male subjects.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

1 Assmann, G. & Gotto, A. M. Jr HDL cholesterol and protective factors in atherosclerosis. *Circulation* **109**, III8–III14 (2004).
2 Pekkanen, J., Linn, S., Heiss, G., Suchindran, C. M., Leon, A., Rifkind, B. M. *et al.* Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N. Engl. J. Med.* **322**, 1700–1707 (1990).
3 Teramoto, T., Ohashi, Y., Nakaya, N., Yokoyama, S., Mizuno, K. & Nakamura, H. Practical risk prediction tools for coronary heart disease in mild to moderate hypercholesterolemia in Japan: originated from the MEGA study data. *Circ. J.* **72**, 1569–1575 (2008).
4 Heo, S. G., Hwang, J. Y., Uhmn, S., Go, M. J., Oh, B., Lee, J. Y. *et al.* Male-specific genetic effect on hypertension and metabolic disorders. *Hum. Genet.* **133**, 311–319 (2014).
5 Kim, Y. J., Go, M. J., Hu, C., Hong, C. B., Kim, Y. K., Lee, J. Y. *et al.* Large-scale genome-wide association studies in east Asians identify new genetic loci influencing metabolic traits. *Nat. Genet.* **43**, 990–995 (2011).
6 Weterman, M. A., Barth, P. G., van Spaendonck-Zwarts, K. Y., Aronica, E., Poll-The, B. T., Brouwer, O. F. *et al.* Recessive MYL2 mutations cause infantile type I muscle fibre disease and cardiomyopathy. *Brain* **136**, 282–293 (2013).
7 Campuzano, O., Alcalde, M., Berne, P., Castro, V., Guzzo, G., Iglesias, A. *et al.* Genetic testing of candidate genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Eur. J. Med. Genet.* **55**, 225–234 (2012).
8 Lee, J. Y., Lee, B. S., Shin, D. J., Park, K. W., Shin, Y. A., Kim, K. J. *et al.* A genome-wide association study of a coronary artery disease risk variant. *J. Hum. Genet.* **58**, 120–126 (2013).
9 Jee, S. H., Suh, I., Kim, I. S. & Appel, L. J. Smoking and atherosclerotic cardiovascular disease in men with low levels of serum cholesterol: the Korea Medical Insurance Corporation Study. *JAMA* **282**, 2149–2155 (1999).
10 Suh, I., Jee, S. H., Kim, H. C., Nam, C. M., Kim, I. S. & Appel, L. J. Low serum cholesterol and haemorrhagic stroke in men: Korea Medical Insurance Corporation Study. *Lancet* **357**, 922–925 (2001).
11 Poetter, K., Jiang, H., Hassanzadeh, S., Master, S. R., Chang, A., Dalakas, M. C. *et al.* Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat. Genet.* **13**, 63–69 (1996).
12 Macera, M. J., Szabo, P., Wadgaonkar, R., Siddiqui, M. A. & Verma, R. S. Localization of the gene coding for ventricular myosin regulatory light chain (MYL2) to human chromosome 12q23-q24.3. *Genomics* **13**, 829–831 (1992).

13 Hui, L., DelMonte, T. & Ranade, K. Genotyping using the TaqMan assay. *Curr. Protoc. Hum. Genet.* (e-pub ahead of print 1 January 2008; doi:10.1002/0471142905. hg0210s56).

14 Go, M. J., Hwang, J. Y., Kim, Y. J., Oh, J. H., Kim, Y. J., Kwak, S. H. *et al.* New susceptibility loci in MYL2, C12orf51 and OAS1 associated with 1-h plasma glucose as predisposing risk factors for type 2 diabetes in the Korean population. *J. Hum. Genet.* **58**, 362–365 (2013).

15 Qiu, Y. H., Deng, F. Y., Li, M. J. & Lei, S. F. Identification of novel risk genes associated with type 1 diabetes mellitus using a genome-wide gene-based association analysis. *J. Diabetes Investig.* **5**, 649–656 (2014).

16 Matsuo, H., Yamamoto, K., Nakaoka, H., Nakayama, A., Sakiyama, M. & Chiba, T. *et al.* Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. *Ann. Rheum. Dis.* (e-pub ahead of print 2 February 2015; doi:10.1136/annrheumdis-2014-206191).

17 Wen, W., Zheng, W., Okada, Y., Takeuchi, F., Tabara, Y., Hwang, J. Y. *et al.* Meta-analysis of genome-wide association studies in East Asian-ancestry populations identifies four new loci for body mass index. *Hum. Mol. Genet.* **23**, 5492–5504 (2014).

18 Pyun, J. A., Kim, S., Park, K., Baik, I., Cho, N. H., Koh, I. *et al.* Interaction effects of lipoprotein lipase polymorphisms with lifestyle on lipid levels in a Korean population: a cross-sectional study. *Genomics Inform.* **10**, 88–98 (2012).

19 Mo, X., Liu, X., Wang, L., Lu, X., Chen, S., Li, H. *et al.* Association of lipoprotein lipase polymorphism rs2197089 with serum lipid concentrations and LPL gene expression. *J. Hum. Genet.* **58**, 160–164 (2013).

20 Howarth, F. C., Qureshi, M. A., Hassan, Z., Al Kury, L. T., Isaev, D., Parekh, K. *et al.* Changing pattern of gene expression is associated with ventricular myocyte dysfunction and altered mechanisms of Ca2+ signalling in young type 2 Zucker diabetic fatty rat heart. *Exp. Physiol.* **96**, 325–337 (2011).

21 Claes, G. R., van Tienen, F. H., Lindsey, P., Krapels, I. P., Helderman-van den Enden, A. T., Hoos, M. B. *et al.* Hypertrophic remodelling in cardiac regulatory myosin light chain (MYL2) founder mutation carriers. *Eur. Heart J.* (e-pub ahead of print 24 October 2015).

22 LopezJimenez, N., Gerber, S., Popovici, V., Mirza, S., Copren, K., Ta, L. *et al.* Examination of FGFRL1 as a candidate gene for diaphragmatic defects at chromosome 4p16.3 shows that Fgfrl1 null mice have reduced expression of Tpm3, sarcomere genes and Lrtm1 in the diaphragm. *Hum. Genet.* **127**, 325–336 (2010).

23 Sheikh, F., Lyon, R. C. & Chen, J. Functions of myosin light chain-2 (MYL2) in cardiac muscle and disease. *Gene* **569**, 14–20 (2015).

24 Thaiparambil, J. T., Eggers, C. M. & Marcus, A. I. AMPK regulates mitotic spindle orientation through phosphorylation of myosin regulatory light chain. *Mol. Cell. Biol.* **32**, 3203–3217 (2012).