Associations of Common Genetic Variants on *IL-17* Genes and Carotid Intima-Media Thickness

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Aim: Atherosclerosis is a chronic inflammatory process of the arterial wall and carotid intima-media thickness (cIMT) is regarded as its early marker. Several members of the *IL-17* family are involved in pro-inflammatory functions. The specific aim of the study was to explore the relationships of common genetic variants on *IL-17* genes with cIMT thickening.

Methods: In the discovery stage, 146 SNPs on 11 *IL-17* genes were screened for their relationships with cIMT by a case-control study that enrolled 284 and 464 subjects who had thicker and normal cIMT, respectively. Findings were replicated by an independent case-control study that enrolled 282 subjects who had thicker cIMT and 282 age-sex-matched subjects who had normal cIMT.

Results: Among 134 eligible SNPs in the discovery study, only *IL-17RC* rs279545 was significantly correlated with cIMT ($p=6.9 \times 10^{-5}$). The rs279545 and 2 nearby linked SNPs rs55847610 and rs3846167 were included in the validation study. We found that the rs279545*G, rs55847610*G, and rs3846167*C were correlated with significantly higher likelihoods of having thicker cIMT. The corresponding multivariate-adjusted ORs were 1.462 (95% CI: 1.055–2.027), 1.481 (95% CI: 1.090–2.013), and 1.589 (95% CI: 1.147–2.200), respectively. Analyses of rs279545-rs55847610 haplotypes showed that the multivariate-adjusted OR for A-A haplotype was significantly decreased (OR=0.665, 95% CI: 0.487–0.908) and for G-G haplotype was significantly increased (OR=1.539, 95% CI: 1.097–2.161).

Conclusions: We first correlated cIMT, a preclinical clinical cardiovascular marker, with *IL-17RC*, the key molecule in the *IL-17* signaling pathway. Our results indicated that *IL-17RC* may play critical role in the development of atherosclerotic diseases.

Key words: Common carotid intima-media thickness, Single nucleotide polymorphism, *IL-17*, Genetic association study, Community-based study

Introduction

Atherosclerosis is one of the major pathologies of cardiovascular diseases (CVDs)¹⁾. It more frequently occurs in aged subjects, accordingly, the global public health impacts of atherosclerosis will continuously increase with the elongation of life expectancy²⁾. To

alleviate the impacts attributable to atherosclerosis, it is essential to identify biomarkers that present at the earlier pathogenic process and have well predictive ability simultaneously and to explore its determinants that will be served as the target of prevention and control.

Among several parameters that had been tested

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to detect changes in the blood vessel structures and their ability to predict the most deleterious clinical manifestations myocardial infarction and stroke, carotid intima-media thickness (cIMT) can be detected easily and non-invasively using B-mode carotid ultrasound^{3, 4}. In addition to myocardial infarction and stroke⁵⁻⁷, thicker cIMT has been strongly correlated with cognitive impairments⁸⁻⁹. Currently, cIMT measurement is widely used to assess the vascular health status of an individual and thicker cIMT is regarded as an intermediate phenotype of atherosclerosis.

Atherosclerosis is a chronic inflammatory process of the arterial wall¹⁰. Recently, a new T helper cell subset that secretes signature cytokine IL-17 was identified¹¹. Several members of the IL-17 family are involved in pro-inflammatory functions¹². The IL-17 family induces downstream responses through binding with cell surface receptors, e.g., IL-17RC is critical to the binding of IL-17A and IL-17F¹³. Additionally, previous twin studies showed that significant proportions of cIMT variations could be explained by genetic factors¹⁴⁻¹⁶. It is reasonable to hypothesize that genetic variants on these *IL-17*-related genes may also contribute to cIMT thickening. Accordingly, the specific aim of the study was to explore the relationships between common genetic variants in *IL-17*-related genes and cIMT.

Materials and Methods

Study Design and Study Subjects

We used two case-control studies to explore the influences of common genetic variants on *IL-17* genes with cIMT. The study subjects of the discovery study were from a community-based cohort enrolled by the Mitochondria-Aging in Northern Taiwan (MAGNET) study¹⁷. From September 2010 to May 2012, 1607 residents aged 40–74 years were enrolled. Twenty-seven subjects were excluded due to the lack of good quality of recorded carotid ultrasound images, and another 40 subjects who had ever been diagnosed with coronary artery diseases and received cardiac catheterization and cardiovascular stent were also excluded, leaving a total of 1539 middle-aged and elderly participants in the MAGNET study. The 75th percentile of the distribution of the mean fall-wall IMT of common carotid arteries (CCA) was 0.70 mm¹⁸. We randomly selected a sample of 284 subjects who had a mean CCA IMT ≥ 0.70 mm as the cases and selected a sample of 464 subjects who had a mean CCA IMT < 0.70 mm as the controls.

The study subjects of the validation study were selected from an ongoing community-based cohort

enrolled residents aged 40–74 years. Among 1198 voluntarily participants who enrolled during May 2014 and Dec 2016, 1164 of them had good quality of recorded carotid ultrasound images and had no CVD history. We used a 1:1 frequency-matched case-control study design and selected a random sample of 282 subjects from those who had thicker cIMT as the case group. Based on the age (40–49, 50–59, and ≥ 60 years) and sex distribution of the cases, we performed stratified random sampling and selected a total of 282 subjects who had normal cIMT as the controls.

All participants of the two community-based studies voluntarily provided informed consent. The studies complied with the 1975 Helsinki Declaration on ethics in medical research and was reviewed and approved by the Institution Review Board of Mackay Memorial Hospital (14MMHIS075).

Measurements of Anthropometric and Cardiovascular Profiles

Measurements of anthropometric had been described previously¹⁷. In short, body weight and height were measured by a digital system (BW-2200; NAGATA Scale Co. Ltd., Tainan, Taiwan). Waist circumference (WC) was measured at the level of mid-distance between the bottom of the rib cage and the top of the iliac crest. Body mass index (BMI) was calculated as (body weight)/(body height)² (kg/m²). Blood pressure was measured three times by a digital system (UDEXTwin; ELK Co., Daejeon, Korea) in the morning after 10 min of rest. The averages of three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were used for analyses.

A 10 ml of fasting venous blood samples were collected for cardiovascular profile analyses. The levels of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting triglycerides (FTG), and fasting plasma glucose (FPG) were determined by an autoanalyzer (Toshiba TBA c16000; Toshiba Medical System, Holliston, MA, USA) with commercial kits (Denka Seiken, Tokyo, Japan). In this study, hypertension was defined as SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or a history of taking antihypertensive medications. Dyslipidemia was defined as total cholesterol ≥ 240 , LDL-C ≥ 160 , or HDL-C < 40 mg/dL or the use of lipid lowering medicines. Diabetes mellitus (DM) was defined as FPG ≥ 126 mg/dL or the use of hypoglycemic agents. Cigarette smoking was defined as having smoked cigarette for at least 4 days per week and lasting for 3 months or more.

The cIMT Measurement

The measurement of cIMT was performed according to the protocol recommended by the American Society of Echocardiography¹⁹⁾ and as described previously¹⁸⁾. In short, two experienced technicians, who were blind to patients' clinical characteristics, obtained and digitally stored both left and right CCA images by using high-resolution B-mode ultrasonography systems (GE Healthcare Vivid 7 and Vivid E9; General Electric Company, Milwaukee, USA), equipped with a multi-frequency linear array transducer. A well-trained technician measured the far wall IMTs blindly by using automatic contouring software (GE Healthcare EchoPAC version 112.0.2; General Electric-Vingmed, Horten, Norway). The IMT was defined as the distance between the lumen-intima and media-adventitia interfaces and included plaques. The average, minimum, and maximum IMTs of the distal 1–2 cm of the left and right CCA were recorded. Mean cIMT was calculated as the mean of the left and right average IMTs and was used for statistical analyses. Our previous study showed that the measurement of cIMT was highly reliable, with intra-class correlation coefficient r greater than 0.97¹⁸⁾.

SNP Selections

In the discovery study, we used a plate that was designed for the Han Chinese population to screen the relationships of common genetic variants on 11 *IL-17*-related genes with cIMT. There were 146 SNPs within ± 25 Kb of the *IL-17A* ($n=14$), *IL-17B* ($n=11$), *IL-17C* ($n=3$), *IL-17D* ($n=4$), *IL-17E* ($n=15$), *IL-17F* ($n=18$), *IL-17RA* ($n=25$), *IL-17RB* ($n=20$), *IL-17RC* ($n=2$), *IL-17RD* ($n=28$), and *IL-17RE* ($n=7$) genes. The eligibility of SNPs for association analyses were a call rate $>95\%$, a p -value of Hardy–Weinberg Equilibrium test in the controls >0.001 , and a minor allele frequency $>5\%$. For SNPs with a p -value less than the pre-set significance level, calculated as $0.05/146=3.4 \times 10^{-4}$, were regarded as candidate genetic markers and were subjected to validation study.

In addition to the candidate SNP, SNPs that are highly linked with candidate genetic variants and may influence expression and regulation of the associated *IL-17* genes were considered for the validation study. The linkage disequilibrium (LD) data in the 1000 Human Genome Project Phase 3-Southern Han Chinese²⁰⁾ were retrieved by using the Ensemble Genome Browser²¹⁾. The cut-off LD (r^2) value of linkage was set at 0.80.

Genotyping

Genomic DNA of each subject was extracted

from EDTA-containing whole blood samples by a semi-automated extraction system Smart LabAssist (Taiwan Advanced Nanotech Inc., Tau-Yuan County, Taiwan) with TANBead Blood DNA plate (Taiwan Advanced Nanotech Inc.).

We used the Axiom® CHB 1 Array Plate (Affymetrix Ltd, Santa Clara, CA, USA) (Affymetrix Ltd) and the Sequenom iPLEX MassARRAY system (Sequenom, San Diego, CA) to determine the genotypes of study subjects of the discovery and validation studies, respectively. All genotyping were performed by the National Center for Genome Medicine, Academic Sinica, Taiwan.

Statistical Analyses

In the study, we used the student's t and the chi-square tests to compare whether there were significant differences in the anthropometric and laboratory measurements between cases and controls. The chi-square test was also used to compare whether there were significant differences in the frequency distributions of genotypes among groups. The Z test was used to compare the significance of relative frequencies of minor alleles between case and control groups.

The relationships with thicker cIMT for common genetic variants on *IL-17* genes were assessed by the additive genotypic effect model. Anthropometric and clinical factors significantly correlated with cIMT were subject to multivariate logistic regression. Assessment of pairwise LD and estimation of haplotype frequencies were performed by Haploview 4.2 software²²⁾. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated by models with and without clinical markers. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Clinical Characteristics of Study Subjects

In the discovery study, cases had significantly higher means of all, except for HDL-C, anthropometric and laboratory measurements than those of the controls (Table 1). The mean level of HDL-C and the proportion of female subjects were significantly lower in the cases than that of the controls. As compared with the controls, the prevalence rates of dyslipidemia, hypertension, and DM were significantly higher in the case group. In the validation study, similar differences in all, except for age, sex, and DM, anthropometric and laboratory measurements between cases and controls were observed.

Among 146 screened SNPs, 12 of them were excluded due to inadequate call rate ($n=4$), a minor

Table 1. Clinical characteristics of subjects of the discovery and validation studies

Variable	Discovery study				Validation study			
	Controls (N=464)		Cases (n=284)		Controls (N=282)		Cases (n=282)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age at enrollment (years)	52.6	8.7	58.9	8.8	58.0	8.5	58.5	8.5
BMI (kg/m ²)	24.1	3.5	25.9	3.3	24.4	3.6	25.7	3.2
Waist circumference (cm)	80.1	9.5	84.9	8.4	81.9	9.5	83.8	8.6
Hip circumference (cm)	94.5	9.2	96.5	6.2	94.4	6.9	96.4	6.1
Waist-to-hip ratio (%)	84.9	7.4	88.0	6.4	86.7	7.1	86.9	6.1
SBP (mm Hg)	126.1	19.5	135.0	17.7	128.6	19.5	135.2	18.2
DBP (mm Hg)	78.6	13.3	82.6	13.4	79.7	14.6	82.3	13.8
Total cholesterol (mg/dL)	205.7	36.0	216.2	42.6	208.9	38.3	213.7	37.9
LDL-C (mg/dL)	121.4	33.2	133.8	35.8	123.2	35.5	131.6	31.3
HDL-C (mg/dL)	56.9	16.0	52.0	14.4	56.5	16.4	53.1	14.5
Fasting triglyceride (mg/dL)	115.9	97.3	132.3	81.8	121.7	106.2	123.4	76.9
Fasting plasma glucose (mg/dL)	97.1	25.9	108.7	36.0	101.7	29.2	105.0	32.3
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Male sex	213	45.9	157	55.3	143	50.0	143	50.0
Cigarette smoking	74	16.1	52	18.6	38	13.5	48	17.0
Dyslipidemia	124	26.7	133	46.8	90	31.9	121	42.9
Diabetes mellitus	25	5.4	39	13.7	29	10.3	34	12.1
Hypertension	134	28.9	134	47.4	93	33.0	128	45.4

allele frequency of <0.05 ($n=3$), and significant deviation from Hardy–Weinberg equilibrium (P value of $<1 \times 10^{-3}$; $n=5$). Among the 134 eligible SNPs for the association analyses, only rs279545 passed the pre-set significance level of the discovery study and was regarded as candidate genetic marker (**Supplementary Table 1**).

The LD data of the Southern Han Chinese shows that 5 SNPs, including rs3774207, rs55847610, rs59465469, rs55847233, and rs3846167, fitted to the inclusion criteria of the validation study. The rs3774207, rs55847610, rs59465469, and rs55847233 are of the same complete LD block²⁰), and among them, rs55847610 is the most suitable for the genotyping platform. Therefore, rs279545, rs55847610, and rs3846167 were included in the validation study (**Supplementary Table 2**).

The test statistics showed that the distributions of the rs279545, rs55847610 and rs3846167 genotypes were significantly different between the case and control groups (**Table 2**). The minor alleles, i.e., rs279545*G, rs55847610*G, and rs3846167*C, were correlated with higher likelihoods of having thicker cIMT. Further adjustment for age, sex, and traditional CVD risk factors, the estimated ORs of having thicker cIMT changed slightly. The corresponding multivari-

ate-adjusted ORs were 1.462 (95% CI: 1.055–2.027), 1.481 (95% CI: 1.090–2.013), and 1.589 (95% CI: 1.147–2.200), respectively.

LD analyses showed that the value of D prime between rs279545 and rs55847610 was 0.96 and was 0.81 for rs279545 with rs3846167. The rs279545 and rs55847610 were grouped as a LD block. We subsequently analyzed the relationship of rs279545-rs55847610 haplotype with the risk of thicker cIMT (**Table 3**). The multivariate-adjusted OR was significantly decreased for A-A haplotype (OR=0.665, 95% CI: 0.487–0.908; $p=0.010$) and significantly increased for G-G haplotype (OR=1.539, 95% CI: 1.097–2.161; $p=0.013$).

Discussion

In the present study, we first identified rs279545 of the *IL-17RC* gene as a promising genetic marker of cIMT thickening and confirmed the finding by subsequent validation study. After adjustment for traditional CVD risk factors, OR of having thicker cIMT for the rs279545-G allele remained significantly increased. In addition to rs279545, two closely linked SNPs rs55847610 and rs3846167 also demonstrating significant correlations with cIMT thickening, which strengthen the validity of our findings. To our knowl-

Table 2. Association analyses for 3 candidate SNPs with thicker carotid IMT

SNP	Allele A/B ¹	Thicker cIMT		Normal cIMT		P _{GT}	P _{TR}	Crude OR OR (95% CI)	Adjusted OR ² OR (95% CI)
		B%	AA/AB/BB	B%	AA/AB/BB				
Discovery study									
rs279545	A/G	22.9	172/94/18	14.8	333/125/6	5.7 × 10 ⁻⁵	6.9 × 10 ⁻⁵	1.716** (1.305-2.256)	1.631** (1.201-2.216)
Validation study									
rs279545	A/G	20.2	185/80/17	13.5	209/70/3	0.003	0.003	1.595** (1.166-2.182)	1.462* (1.055-2.027)
rs55847610	A/G	23.2	171/91/20	16.0	198/78/6	0.005	0.003	1.565** (1.165-2.102)	1.481* (1.090-2.013)
rs3846167	T/C	20.2	185/77/18	12.9	211/64/4	0.003	0.002	1.643** (1.201-2.249)	1.589* (1.147-2.200)

¹, Allele A/B, major/minor alleles.², OR were adjusted for age, sex, SBP, LDL-C, BMI, and cigarette smoking.Note: cIMT, carotid intima-media thickness; CI, confidence interval; OR, odds ratio; P_{GT}, *p*-value for the Chi-square test of genotype distribution between subjects who had thicker and normal cIMT; P_{TR}, *p*-value for the additive genotypic model.*, 0.005 < *P* < 0.05; **, *P* < 0.005.**Table 3.** Association analyses for rs279545-rs55847610 haplotypes with thicker carotid IMT

rs279545-rs55847610 haplotype	Controls 2n (%)	Cases 2n (%)	Crude OR (95% CI)	Multivariate-adjusted OR ¹ (95% CI)
A-A	475 (84.2)	433 (76.8)	0.619** (0.459-0.835)	0.665* (0.487-0.908)
G-G	71 (12.6)	108 (19.1)	1.645** (1.188-2.277)	1.539* (1.097-2.161)
A-G	18 (3.2)	23 (4.1)	1.290 (0.688-2.417)	1.214 (0.631-2.335)

¹, OR were adjusted for age, sex, SBP, LDL-C, BMI, and cigarette smoking.

Note: cIMT, carotid intima-media thickness; CI, confidence interval; OR, odds ratio.

*, 0.005 < *P* < 0.05; **, *P* < 0.005.

edge, there was no report correlated genetic polymorphism of *IL-17RC* with a preclinical CVD marker. Our findings provided potential pathophysiological targets and clinical biomarkers for CVD treatments and predictions.

The indexed SNP rs279545 is located on the genic region of *IL-17RC* and is a non-coding transcript variant²⁰. To date, there were only 2 published reports about its putative functions. The rs279545 was the lead SNP that linked with local adaptation for eastern Asians²³ and was involved in the actin cytoskeleton pathway showing significant association with basal cell carcinoma²⁴. To explore possible function related to rs279545 polymorphism, we searched the Taiwan Biobank to identify variants that result in functional change and has a minor allele frequency > 3% in the *IL-17RC*. We found only one missense SNP rs708567 fits the criteria²⁵. However, LD data of the Southern Han Chinese showed that this variant was not linked with the rs279545 polymorphism but in complete LD with the rs279590 polymorphism²⁰. Notably, the discovery study did not correlate rs279590 polymorphism with cIMT (**Supplementary Table 1**). Accordingly, the rs708567 polymorphism

was unlikely a genetic marker for cIMT thickening. The role of the rs279545 polymorphism in the regulation and expression of *IL-17RC* waits for exploration.

Although there was no report exploring the effect of *IL-17RC* on atherosclerosis, a few human studies had explored the atherogenic effects of *IL-17A*, the ligand of *IL-17RC*. Initially, Li *et al.* (2005) assayed the *IL-17* levels in the ischemic hemisphere of the human brain by an immunochemistry method²⁶. They found that *IL-17* levels were elevated in the ischemic hemispheres of human brains and peaked at days 3–5 after brain ischemia. Furthermore, only very few *IL-17*-positive cells were observed in the opposite normal hemispheres of human brains²⁶. Similarly, several research groups observed *IL-17A* were expressed in human atherosclerotic lesions²⁷⁻³¹ and correlated the *IL-17A* expression levels with the vulnerability of atherosclerotic lesions^{28, 29}. On the contrarily, one study reported that *IL-17A* expression was associated with more stable plaque phenotype rather than macrophage rich plaque areas³².

In addition to *IL-17* expression studies, there were studies compared the frequency of Th17 cells and levels of circulating *IL-17* between patients with

atherosclerotic diseases and controls. Most of flow cytometric studies showed that Th17 frequency in peripheral blood, either alone or in relation to T-cell count, was elevated in unstable or more advanced atherosclerosis^{28, 29, 32-37}. However, Th17 cell was not found in atherosclerotic tissues³⁸ and as compared with patients with acute myocardial infarction, the frequency of Th17 cells was significantly higher in patients with stable angina³⁹. Similarly, studies of serum or plasma levels of circulating IL-17A and severity or vulnerability of atherosclerosis showed inconsistent results. Most of studies had shown higher levels of circulating IL-17A in subjects with unstable or more advanced plaques³¹⁻³⁶. Unexpectedly, a prospective, multicenter study that enrolled acute ST-elevation and non-ST-elevation MI patients found that lower serum IL17 levels correlated with significantly higher risks of all-cause mortality and recurrent MI⁴⁰.

Collectively, some human studies suggested that IL-17 may promote the development of atherosclerosis while others pointed to IL-17 as an anti-atherogenic factor. Similarly, studies of isolated cells and animals demonstrated inconsistent findings^{41, 42}. The inconsistent findings of different types of studies implicate that the involvement of IL-17 in atherosclerosis is complex. Due to the IL-17 signaling depend on the binding of IL-17s with cell surface receptors IL-17Rs, attempt to explore the effects of IL-17RC variants may help to clarify those discrepancies. Our findings provide a notch for further research.

There were potential limitations of the study. Our pretest showed that high proportion of subjects affected with hyperlipidemia, DM, and hypertension were not able to recall the date of diagnosis and provide information associated with treatments. Therefore, we did not collect these data and were not able to assess the effects of treatments on cIMT in the study. Theoretically, genotypes are not correlated with receiving treatment or not, accordingly, our findings were unlikely results of confounding. Additionally, all study subjects in the discovery and validation studies were restricted to Han Chinese, which may limit the external validity of the study.

In conclusion, we were first to successfully correlate cIMT, a preclinical clinical CVD marker, with IL-17RC, the key molecule in the IL-17 signaling pathway. Our results indicated that IL-17RC may play critical role in the development of atherosclerotic diseases.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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Supplementary Table 1. SNPs within 25 Kb up- and down-stream of the *IL-17* genes in the discovery study

GENE	SNP	CHR	Position GRCh37. p13	Allele A/B	Thicker cIMT (<i>n</i> =284)		Normal cIMT (<i>n</i> =464)		P _{HWE}	P _{GT}	P _{TR}
					Allele A %	AA/AB/BB	Allele A %	AA/AB/BB			
IL17RE	rs279543	3	9935263	AG	91.4	236/47/1	92.3	395/67/2	6.4E-01	7.3E-01	4.9E-01
	rs33970214	3	9939361	AG	25.3	18/107/158	24.1	27/170/267	9.9E-01	8.9E-01	6.2E-01
	rs11706730	3	9939620	AG	65.7	117/139/28	67.3	216/193/55	2.4E-01	1.4E-01	5.0E-01
	rs783493	3	9940833	AT	91.4	236/47/1	92.3	395/67/2	6.4E-01	7.3E-01	4.9E-01
	rs279590	3	9943072	AG	91.2	237/44/3	91.8	392/68/4	5.8E-01	9.2E-01	6.8E-01
IL17RC	rs279545	3	9972493	AG	77.1	172/94/18	85.2	333/125/6	1.3E-01	5.7E-05	6.9E-05
	rs3894571	3	9977244	TC	57.1	99/125/59	58.7	159/227/78	8.4E-01	3.0E-01	5.3E-01
IL17RB	rs2241807	3	53857158	TC	57.2	96/133/55	58.5	153/237/74	2.6E-01	3.9E-01	6.2E-01
	rs74929132	3	53867649	AG	11.4	4/57/223	8.9	3/77/384	6.8E-01	2.6E-01	1.2E-01
	rs930367	3	53873578	TC	69.0	135/122/27	67.8	223/183/58	3.7E-02	3.8E-01	6.3E-01
	rs1025689	3	53883722	CG	53.7	85/135/64	53.3	136/223/105	4.6E-01	9.8E-01	9.0E-01
	rs55754695	3	53884914	AC	87.9	218/63/3	85.7	340/115/9	8.4E-01	4.4E-01	2.3E-01
	rs3774618	3	53885173	CG	48.8	71/135/78	49.6	122/216/126	1.4E-01	9.2E-01	7.7E-01
	rs3733075	3	53886912	TC	61.8	110/130/43	61.5	171/229/64	3.6E-01	6.5E-01	9.0E-01
	rs6766099	3	53894412	TC	32.6	34/117/133	32.0	48/201/215	9.2E-01	7.3E-01	8.2E-01
	rs12637033	3	53896850	TC	82.4	192/84/8	85.8	339/118/7	3.6E-01	1.9E-01	7.5E-02
	rs2232350	3	53899178	TC	82.0	190/86/8	85.3	336/120/8	4.7E-01	2.2E-01	8.5E-02
	rs4687756	3	53904911	TC	62.3	112/130/42	59.9	163/230/71	4.9E-01	4.9E-01	3.5E-01
	rs12632292	3	53906951	AG	42.8	56/131/97	41.4	74/236/154	3.0E-01	3.2E-01	5.9E-01
	rs3733079	3	53911540	AG	44.9	61/132/90	47.4	100/240/124	4.2E-01	2.9E-01	3.4E-01
	rs2276845	3	53916130	AG	54.9	90/132/62	52.6	124/240/100	4.2E-01	2.9E-01	3.8E-01
	rs74447517	3	53918316	AG	87.8	216/63/3	88.9	365/95/4	4.2E-01	7.9E-01	5.0E-01
	rs55835925	3	53919080	TG	88.9	223/59/2	88.9	366/93/5	7.4E-01	8.6E-01	1.0E+00
IL17RD	rs1386830	3	57099775	TG	44.5	53/145/84	44.9	90/237/137	4.9E-01	9.8E-01	8.7E-01
	rs11717604	3	57103631	TC	38.9	47/127/110	40.1	71/229/163	5.2E-01	4.5E-01	6.6E-01
	rs7427326	3	57104606	AG	84.3	199/81/4	85.1	336/118/10	9.2E-01	5.2E-01	6.7E-01
	rs78227129	3	57105813	AG	60.7	109/127/48	59.6	163/226/74	7.7E-01	5.5E-01	6.7E-01
	rs12636899	3	57108551	AC	52.5	81/135/67	51.6	125/227/110	7.3E-01	8.9E-01	7.5E-01
	rs1463657	3	57137461	AC	13.4	2/72/210	11.2	6/92/365	9.4E-01	1.7E-01	2.1E-01
	rs1545982	3	57139833	AG	47.3	67/134/82	47.3	104/231/129	9.8E-01	8.1E-01	9.9E-01
	rs1545981	3	57139972	AG	52.1	80/136/68	51.4	123/230/110	9.0E-01	8.7E-01	7.9E-01
	rs1872946	3	57149229	TC	13.4	2/72/210	12.0	8/95/361	5.5E-01	1.7E-01	4.2E-01
	rs12487790	3	57149659	TC	13.4	2/72/210	12.0	8/95/361	5.5E-01	1.7E-01	4.2E-01
	rs747089	3	57153869	TC	34.3	36/123/125	36.5	58/222/183	4.6E-01	4.3E-01	3.9E-01
	rs79385923	3	57162471	AG	16.6	10/72/195	15.5	19/104/335	4.4E-03	5.8E-01	5.9E-01
	rs6796041	3	57163887	TG	35.2	35/130/119	37.1	62/220/182	7.3E-01	7.6E-01	4.7E-01
	rs7374667	3	57173915	TC	42.3	56/128/100	43.4	82/238/143	3.2E-01	2.4E-01	6.6E-01
	rs6801574	3	57177195	TC	51.6	75/142/66	52.2	122/239/102	4.6E-01	9.0E-01	8.3E-01
	rs6808185	3	57179261	CG	65.2	120/129/34	63.1	184/218/62	8.4E-01	7.2E-01	4.2E-01
	rs75205515	3	57182663	AG	34.7	34/129/121	37.4	62/223/179	5.7E-01	5.4E-01	2.9E-01
	rs17216900	3	57183877	TC	48.8	68/141/75	47.2	101/236/127	6.6E-01	7.9E-01	5.5E-01
	rs4475007	3	57186159	AG	62.7	112/132/40	57.9	149/239/76	2.3E-01	1.2E-01	6.2E-02
	rs17235841	3	57186580	AC	81.0	184/92/8	83.8	322/134/8	1.6E-01	3.2E-01	1.4E-01
	rs17289049	3	57187036	AG	66.2	124/128/32	63.3	182/223/59	4.7E-01	4.8E-01	2.4E-01
	rs11710921	3	57192294	TG	17.3	6/86/192	14.5	7/121/336	2.9E-01	3.5E-01	1.5E-01
	rs74370320	3	57202491	TC	5.3	3/24/257	3.6	0/33/431	4.3E-01	6.6E-02	1.2E-01
	rs4455299	3	57204445	CG	49.6	67/148/69	49.7	114/233/117	9.3E-01	8.8E-01	9.9E-01
	rs6445860	3	57209197	TG	94.0	251/32/1	94.7	416/47/1	7.9E-01	8.3E-01	5.6E-01
	rs7640279	3	57221834	AC	93.5	247/37/0	94.7	418/43/3	1.1E-01	1.1E-01	3.2E-01

(Cont Supplementary Table 1)

GENE	SNP	CHR	Position GRCh37. p13	Allele A/B	Thicker cIMT (<i>n</i> =284)		Normal cIMT (<i>n</i> =464)		P _{HWE}	P _{GT}	P _{TR}
					Allele A %	AA/AB/BB	Allele A %	AA/AB/BB			
IL17B	rs74577786	5	148742739	TC	8.5	1/46/237	6.4	1/57/404	4.9E-01	3.1E-01	1.3E-01
	rs62378147	5	148744254	AG	90.8	234/48/2	90.2	381/75/8	6.3E-02	4.9E-01	6.8E-01
	rs353255	5	148748728	AG	22.5	13/102/169	22.2	22/162/280	8.2E-01	9.6E-01	8.8E-01
	rs353278	5	148767903	TC	7.6	2/39/243	8.8	4/74/386	8.3E-01	6.9E-01	3.9E-01
	rs6864982	5	148771975	TC	16.0	6/79/199	12.6	3/111/349	6.4E-02	8.9E-02	5.8E-02
	rs353265	5	148775566	AG	60.4	106/131/47	61.5	169/233/62	1.9E-01	3.9E-01	6.6E-01
	rs353266	5	148776470	TG	16.7	12/71/201	18.5	20/132/312	2.1E-01	5.8E-01	3.9E-01
	rs10054004	5	148779127	AG	16.9	7/82/195	12.9	6/108/350	4.7E-01	9.7E-02	3.1E-02
	rs422727	5	148779512	AG	41.4	53/129/102	37.4	66/215/183	8.2E-01	2.5E-01	1.3E-01
	rs428690	5	148780028	AG	90.1	228/56/0	93.4	404/59/1	4.5E-01	2.7E-02	1.7E-02
IL17A	rs10515627	5	148780713	TC	73.9	156/108/20	74.8	257/176/28	7.7E-01	8.7E-01	7.0E-01
	rs9463765	6	52030644	AG	52.5	71/153/57	54.0	133/233/96	7.4E-01	5.1E-01	5.6E-01
	rs6926641	6	52039396	CG	12.9	2/69/211	13.6	8/110/346	8.3E-01	5.0E-01	7.2E-01
	rs10484880	6	52040594	AG	7.0	2/36/246	9.5	5/78/381	6.5E-01	2.6E-01	1.1E-01
	rs4711998	6	52050353	AG	76.6	168/99/17	74.5	257/176/30	9.9E-01	6.2E-01	3.7E-01
	rs8193036	6	52050493	TC	26.0	21/105/157	27.5	32/191/241	4.8E-01	5.5E-01	5.2E-01
	rs3819024	6	52050786	AG	47.9	74/124/86	49.2	114/228/121	7.5E-01	3.0E-01	6.2E-01
	rs3819025	6	52051274	AG	19.5	13/85/186	18.2	10/149/305	9.3E-02	1.6E-01	5.2E-01
	rs8193040	6	52056093	TG	59.0	109/117/58	55.7	136/245/83	1.3E-01	7.0E-03	2.2E-01
	rs74755742	6	52056934	CG	52.1	85/126/73	54.8	130/246/86	1.1E-01	2.8E-02	3.2E-01
IL17F	rs4601118	6	52063480	AC	28.8	21/120/140	31.2	36/217/210	4.9E-02	4.9E-01	3.1E-01
	rs4715286	6	52066558	TC	28.8	22/119/142	31.1	36/217/211	5.1E-02	4.3E-01	3.2E-01
	rs73739250	6	52068566	TC	86.8	210/68/3	84.5	328/125/9	4.6E-01	4.2E-01	2.1E-01
	rs74296155	6	52068641	AC	52.8	86/126/70	54.8	131/244/87	1.6E-01	6.1E-02	4.7E-01
	rs591274	6	52085748	AG	22.7	11/104/163	23.4	20/173/262	2.0E-01	9.4E-01	7.3E-01
	rs555416	6	52085800	TC	12.5	3/65/216	15.3	10/122/332	7.6E-01	2.8E-01	1.3E-01
	rs11966760	6	52087034	TG	52.1	85/126/73	54.8	132/245/87	1.5E-01	3.6E-02	3.0E-01
	rs2179560	6	52088183	AG	83.8	198/80/6	83.8	320/138/6	3.6E-02	6.4E-01	9.9E-01
	rs13209590	6	52099651	AG	87.1	215/65/4	83.8	325/128/11	7.0E-01	2.1E-01	7.8E-02
	rs763780	6	52101739	TC	85.4	207/71/6	87.3	353/104/7	8.3E-01	5.7E-01	2.9E-01
IL17D	rs12201582	6	52104689	AC	13.3	7/61/215	16.7	20/113/324	1.6E-02	2.1E-01	8.5E-02
	rs9382084	6	52105667	TG	70.6	139/123/22	71.4	235/193/36	6.8E-01	8.9E-01	7.2E-01
	rs117507014	6	52107393	TC	90.1	230/52/2	87.2	352/105/7	7.9E-01	2.1E-01	8.1E-02
	rs607175	6	52112966	TC	59.7	102/135/47	56.1	147/227/90	8.9E-01	4.1E-01	1.8E-01
	rs4715291	6	52113360	TC	17.3	7/84/193	13.9	7/115/342	4.5E-01	2.0E-01	7.4E-02
	rs7741835	6	52118025	TC	29.9	29/112/143	30.2	34/211/217	7.3E-02	1.6E-01	9.1E-01
	rs10948691	6	52118348	AG	42.4	62/117/105	41.9	79/231/154	6.3E-01	5.8E-02	8.5E-01
	rs75637730	6	52118961	AG	83.3	194/85/5	86.0	340/118/6	2.3E-01	3.4E-01	1.4E-01
	rs669161	6	52121015	AG	47.1	66/132/82	45.3	90/234/133	4.8E-01	4.0E-01	4.9E-01
	rs742552	6	52126705	AG	49.8	63/157/64	47.5	112/217/135	1.8E-01	5.8E-02	3.9E-01
IL17E	rs17246626	6	52130312	TG	11.6	5/56/223	15.5	13/118/333	5.2E-01	1.1E-01	3.9E-02
	rs7995773	13	21251235	TC	28.0	14/131/139	30.7	41/203/220	5.5E-01	1.4E-01	2.5E-01
	rs6490605	13	21285009	AG	80.2	178/98/7	76.9	272/167/23	6.8E-01	1.9E-01	1.3E-01
	rs183542740	13	21316496	AG	94.9	254/27/1	96.5	432/30/1	5.3E-01	2.8E-01	1.2E-01
IL17E	rs75484365	13	21316654	TC	95.6	260/23/1	97.3	440/23/1	2.4E-01	2.1E-01	8.2E-02
	rs74704321	14	23820310	AG	90.7	235/45/4	91.5	388/73/3	8.3E-01	5.7E-01	5.9E-01
	rs72542466	14	23821361	AG	8.1	1/43/233	8.2	3/69/387	9.7E-01	8.6E-01	9.7E-01
	rs12433202	14	23822683	AG	65.3	118/135/31	63.1	190/206/68	3.2E-01	3.2E-01	4.0E-01
IL17E	rs7148564	14	23823913	AT	11.8	2/63/219	9.4	2/83/379	2.6E-01	3.1E-01	1.2E-01

(Cont Supplementary Table 1)

GENE	SNP	CHR	Position GRCh37. p13	Allele A/B	Thicker cIMT (<i>n</i> =284)		Normal cIMT (<i>n</i> =464)		P _{HWE}	P _{GT}	P _{TR}
					Allele A %	AA/AB/BB	Allele A %	AA/AB/BB			
IL17E	rs7142405	14	23824114	AG	36.8	36/137/111	31.8	48/199/217	8.1E-01	1.1E-01	4.6E-02
	rs10162489	14	23824536	TC	63.2	113/133/38	62.2	182/213/69	6.0E-01	8.5E-01	6.9E-01
	rs80293125	14	23824799	AC	12.7	2/68/214	10.3	2/91/368	1.4E-01	3.4E-01	1.4E-01
	rs2231811	14	23826792	AG	11.6	2/62/220	9.3	2/82/380	2.7E-01	3.2E-01	1.3E-01
	rs2284652	14	23831177	TC	36.8	36/137/111	31.7	48/198/218	7.6E-01	1.0E-01	4.2E-02
	rs10143597	14	23842739	TG	31.7	33/114/137	29.2	41/189/234	7.5E-01	4.6E-01	3.2E-01
	rs79877597	14	23844979	AC	29.2	26/114/144	27.6	39/178/247	3.9E-01	7.9E-01	5.0E-01
	rs8006357	14	23853629	TC	82.9	197/77/10	81.0	302/146/15	6.0E-01	4.4E-01	3.5E-01
	rs12893772	14	23856381	AC	91.2	236/46/2	90.4	379/81/4	8.9E-01	8.8E-01	6.1E-01
	rs17091434	14	23860708	TG	8.8	4/42/238	9.1	3/78/383	6.5E-01	4.5E-01	8.7E-01
IL17C	rs365990	14	23861811	AG	83.5	201/72/11	81.4	304/147/13	3.4E-01	1.5E-01	3.1E-01
	rs8047318	16	88683946	AG	45.4	64/130/90	42.2	81/230/153	7.3E-01	2.3E-01	2.3E-01
	rs12709102	16	88712319	TC	75.5	160/109/15	73.7	248/188/28	3.3E-01	7.2E-01	4.2E-01
IL17RA	rs6500487	16	88727733	TC	51.9	77/141/66	55.4	133/248/83	7.9E-02	2.0E-01	1.8E-01
	rs4819956	22	17561913	AC	72.9	157/100/27	70.9	227/204/33	1.6E-01	5.1E-02	4.1E-01
	rs2241043	22	17567807	TC	34.2	33/128/123	35.3	57/214/193	8.4E-01	8.9E-01	6.4E-01
	rs5994158	22	17567898	AG	41.2	49/135/99	40.0	69/232/162	3.4E-01	6.5E-01	6.4E-01
	rs9606607	22	17568467	TC	69.1	135/121/27	70.2	233/185/46	3.0E-01	7.4E-01	6.7E-01
	rs5748863	22	17571355	AG	31.5	28/123/133	32.1	47/204/213	8.6E-01	9.7E-01	8.1E-01
	rs5748864	22	17572941	AG	38.4	45/128/111	42.5	82/230/152	7.6E-01	2.1E-01	1.2E-01
	rs1990502	22	17573272	AG	78.0	178/87/19	81.9	317/126/21	7.0E-02	2.1E-01	7.8E-02
	rs6518660	22	17575800	AG	84.3	198/78/5	80.1	296/142/20	5.7E-01	8.4E-02	4.2E-02
	rs9605215	22	17579013	AC	76.6	171/93/20	76.2	267/173/24	5.5E-01	3.2E-01	8.6E-01
	rs2229151	22	17589297	AG	29.9	27/116/141	29.1	33/204/227	1.6E-01	4.3E-01	7.3E-01
	rs887796	22	17593685	AG	83.3	197/79/8	82.1	310/142/12	3.7E-01	7.2E-01	5.6E-01
	rs738035	22	17594886	TC	61.8	108/134/41	63.1	182/222/60	5.5E-01	8.3E-01	6.1E-01
	rs3827278	22	17595915	AC	31.3	29/120/135	31.3	43/204/216	6.0E-01	8.5E-01	9.9E-01
	rs35597091	22	17598813	AG	15.7	7/75/202	15.4	15/113/336	1.6E-01	7.1E-01	9.0E-01
	rs5994165	22	17600977	AG	47.2	67/134/83	46.3	93/242/127	2.5E-01	3.5E-01	7.4E-01
	rs5746996	22	17603801	AC	32.2	29/125/130	33.5	47/217/200	2.9E-01	7.5E-01	6.0E-01
	rs5994166	22	17607894	AT	79.2	177/96/11	78.8	283/165/16	1.7E-01	8.6E-01	8.3E-01
	rs74276301	22	17610214	AG	10.9	4/54/226	10.4	4/88/371	6.3E-01	7.8E-01	7.4E-01
	rs79230318	22	17614422	AG	16.9	14/68/202	20.3	16/156/291	3.8E-01	1.5E-02	1.1E-01
	rs4819559	22	17616510	AC	53.0	72/157/55	55.2	134/244/86	1.7E-01	5.8E-01	3.9E-01
	rs5994176	22	17618164	CG	82.7	197/76/11	84.9	330/128/6	9.8E-02	7.1E-02	2.6E-01
	rs2286954	22	17619368	AG	12.9	9/55/220	14.4	6/122/336	1.7E-01	2.6E-02	3.9E-01

Note: P_{HWE}, *p*-value for the HWE test in subjects who had normal cIMT; P_{GT}, *p*-value for the Chi-square test of genotype distribution between subjects who had thicker and normal cIMT; P_{TR}, *p*-value for the additive genotypic model.

Supplementary Table 2. Details of the primers used in the polymorphism genotyping by MassArray

SNP	GRCh38. p10	Alleles	Type of variant	Primer sequences of PCR	Length of PCR product (bp)	Tm(NN)	Annealing primer
rs279545	3:9930809	G/A	Non coding transcript exon variant	ACGTTGGATGTAGCCGGG AGATATGCATAG ACGTTGGATGAGCCAGGT GTTCCAATATTC	101	46.8	cccgGGCCTGATC TCCAAATT
rs55847610	3:9949479	G/A	Missense	ACGTTGGATGACTGGCCT CTTGACAGTACC ACGTTGGATGACTTCTCC CTCAGACCACCA	91	48.6	CCCCACACCCT TGTT
rs3846167	3:9954742	C/T	Non coding transcript exon variant	ACGTTGGATGGTGGTACA TGAAGAGATGGG ACGTTGGATGATTCATACG GCCTCACACTG	120	45.2	aTTTCTAGCAGC CACAT