

## Research Paper

# IGF1 Gene Is Associated With Triglyceride Levels In Subjects With Family History Of Hypertension From The SAPPHIRe And TWB Projects

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Received: 2018.02.26; Accepted: 2018.05.14; Published: 2018.06.14

## Abstract

Chromosome 12q23-q24 has been linked to triglyceride (TG) levels by previous linkage studies, and it contains the *Insulin-like growth factor 1 (IGF1)* gene. We investigated the association between *IGF1* and TG levels using two independent samples collected in Taiwan. First, based on 954 siblings in 397 families from the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe), we found that rs978458 was associated with TG levels ( $\beta = -0.049$ ,  $p = 0.0043$ ) under a recessive genetic model. Specifically, subjects carrying the homozygous genotype of the minor allele had lower TG levels, compared with other subjects. Then, a series of stratification analyses in a large sample of 13,193 unrelated subjects from the Taiwan biobank (TWB) project showed that this association appeared in subjects with a family history (FH) of hypertension ( $\beta = -0.045$ ,  $p = 0.0000034$ ), but not in subjects without such an FH. A re-examination of the SAPPHIRe sample confirmed that this association appeared in subjects with an FH of hypertension ( $\beta = -0.068$ ,  $p = 0.0025$ ), but not in subjects without an FH. The successful replication in two independent samples indicated that *IGF1* is associated with TG levels in subjects with an FH of hypertension in Taiwan.

Key words: Family history of hypertension; IGF1; Insulin-like growth factor 1; Single-nucleotide polymorphism; Triglyceride

## Introduction

An elevated level of triglyceride (TG) is a risk factor for cardiovascular disease (CVD), and particularly atherosclerotic CVD [1, 2]. The estimated heritability of TG levels ranges from 31% to 52% [3-5], which indicates that genetic factors play important roles in regulating TG levels. Identifications of the genetic determinants of TG could be helpful to the

development of drugs for controlling circulating TG levels as well as risk of CVD [6]. TG levels can be influenced by both rare and common genetic variants. For example, many rare genetic variants within the *LPL*, *APOC2*, *LMF1*, *GPIHBP1*, and *APOA5* genes were shown to strongly affect TG levels [7, 8]. On the other hand, in the past decade, many common

single-nucleotide polymorphisms (SNPs) within more than 40 loci were associated with TG levels through genome-wide association studies (GWASs) [9-14]. Nevertheless, relevant rare and common genetic variants identified to date can explain only a limited proportion of the heritability of TG levels [7]. To look for genetic variants accounting for the missing heritability of TG levels, further efforts are needed.

In addition to GWASs, applying an association analysis to candidate genes obtained from studies of genome-wide linkage scans is another practical approach to identify genes responsible for the variability in TG levels [5]. Many studies of genome-wide linkage scan for TG levels have been conducted [3-5, 15-23], among which, Feitosa et al. (2006) reported a quantitative trait locus for TG on chromosome 12q23-q24 and suggested that insulin-like growth factor-1 (*IGF1*) was a candidate gene [23]. In addition, based on the Chinese families collected from the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRE), Hsiao et al. (2006) also linked this region to TG levels [21]. The *IGF1* gene encodes human IGF1, a circulating polypeptide consisting of 70 amino acids which plays important roles in growth, cell differentiation, and metabolism [24]. Although IGF1 levels were associated with TG levels in several studies [25-27], few studies examined and reported the association between the *IGF1* gene and TG levels [24].

In this study, we investigated the association between the *IGF1* gene and TG levels by using two independent samples collected from Taiwanese populations. First, based on a sample of siblings from Taiwanese families recruited by SAPPHIRE [28], we identified tag-SNPs within *IGF1* and examined their associations with TG levels. Then, we tried to replicate significant associations obtained in the SAPPHIRE sample using a large sample of unrelated subjects collected by the Taiwan biobank (TWB) project [29].

## Materials & Methods

### Study design and study samples

This was a cross-sectional candidate gene association study, and two independent samples collected in Taiwan were used to investigate the associations of common SNPs in *IGF1* with TG levels.

The first study sample was taken from the SAPPHIRE study, which is an international collaborative project to identify genetic determinants influencing susceptibility to hypertension and insulin resistance, based on concordant sibpairs (with both sibs being hypertensive) and discordant sibpairs (with one hypertensive and one hypotensive sib).

Originally, the SAPPHIRE network recruited hypertensive subjects of Chinese or Japanese descent and their family members at six sites in Taiwan, Hawaii, and the California Bay area from 1995 to 2000 [28]. In the current study, data on Taiwanese participants in SAPPHIRE were analyzed.

The second study sample was taken from the TWB project, which is an ongoing project and aims to collect clinical, lifestyle, and genomic data of 300,000 residents of Taiwan [29]. As of the end of May 2016, 15,965 participants with clinical, lifestyle, and genomic data were released by the TWB database. After excluding subjects with self-reported hyperlipidemia and extreme value of TG levels ( $> 7.345$  mmol/L) [30], 14,858 subjects remained. Among which, 13,193 unrelated subjects (defined as the pairwise PI\_HAT statistic  $< 0.06$  in PLINK [31]) were identified and analyzed in this study.

The institutional review boards of the National Health Research Institutes, National Taiwan University Hospital, Taipei Veterans General Hospital, Taichung Veterans General Hospital, and Tri-Service General Hospital approved this study. Informed consent forms were signed by all participants at study entry.

### Clinical measures and lifestyle factors

In the SAPPHIRE study, the participants underwent anthropometric measurements at 08:00 after an 8~10-h overnight fast, and blood samples were collected after the anthropometric measurements were taken. The body mass index (BMI) was defined as the weight in kilograms divided by the square of height in meters ( $\text{kg}/\text{m}^2$ ). The TG level was measured in fasting blood samples. The measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP) was performed after sitting at rest for 10 min, and the number of medications used for controlling high blood pressure was recorded. A SAPPHIRE subject meeting the following criteria was defined as hypertensive: (1) having SBP  $\geq 140$  mmHg or DBP  $\geq 90$  mmHg, or (2) taking at least one medication to control high blood pressure. Lifestyle factors considered in this study included cigarette smoking, alcohol consumption, and physical activity, which were obtained by a questionnaire. The smoking status was dichotomized as a current- or ever-smoker versus a never-smoker. The alcohol-consumption status was dichotomized as a current- or ever-drinker versus a never-drinker. For physical activity, subjects were dichotomized as non-sedentary versus sedentary [32].

Relevant clinical and lifestyle data of TWB subjects were provided by the TWB database. BMI, smoking status, and alcohol-consumption status were

defined the same as those in the SAPPHiRe sample. For each subject, the TWB database provided the measurements of the SBP and DBP, and the self-reported hypertension status, but no information about hypertension medications. Hence, a TWB subject meeting the following criteria was defined as hypertensive: (1) having SBP  $\geq$  140 mmHg or DBP  $\geq$  90 mmHg, or (2) having self-reported hypertension. For each subject, information of the number of hypertensive first-degree relatives was available. For physical activity, subjects were dichotomized as with exercise habits versus without exercise habits.

In both the SAPPHiRe and TWB samples, a subject meeting the following criteria was defined as having a family history (FH) of hypertension: (1) the subject was hypertensive and at least one of his/her first-degree relatives was hypertensive, or (2) the subject was non-hypertensive and at least two of his/her first-degree relatives were hypertensive.

### SNP selection and genotyping

For SAPPHiRe subjects, genomic DNA was extracted from a blood sample by a conventional phenol/chloroform extraction method. SNP information of the *IGF1* gene was obtained by resequencing functional regions (exons, intron-exon boundaries, the promoter region, and the 3' untranslated region) in 24 SAPPHiRe subjects. Genetic variants with a minor allele frequency (MAF)  $>$  5% were prioritized for further study. In total, nine SNPs, including rs2288377, rs2195239, rs978458, rs1520220, rs6220, rs6217, rs6218, rs6214, and rs6219, were selected and genotyped in the SAPPHiRe sample. SNP genotyping was performed using an ABI Prism 7700HT Sequence Detection System (Applied Biosystems) based on the 5' nuclease allelic discrimination (Taqman) assay. Information about these nine SNPs and genotype data of the SAPPHiRe sample are presented in Supplementary Table S1. Genotype data of TWB subjects were provided by the TWB database, which were generated using the TWB genotype array [29].

All methods were implemented in accordance with the approved guidelines and regulations. The experimental protocols used in this study were approved by committees from the National Health Research Institutes, National Taiwan University Hospital, Taipei Veterans General Hospital, Taichung Veterans General Hospital, and Tri-Service General Hospital.

### Statistical analysis

Clinical characteristics of both the SAPPHiRe and TWB samples are given as follows. Quantitative variables, except for TG levels, were expressed as the

mean  $\pm$  standard deviation (SD). The TG level was expressed as the geometric mean with the interquartile range (IQR) because of its positively skewed distribution. Qualitative variables are presented as percentages.

The Haploview software [33] was used to estimate the MAF and test for Hardy-Weinberg equilibrium (HWE) of the SNPs genotyped in the SAPPHiRe sample. Furthermore, the extent of linkage disequilibrium (LD) between any pair of these SNPs was estimated by Haploview, and selection of tag-SNPs was based on a threshold of  $r^2 = 0.8$ .

In each of the SAPPHiRe and TWB samples, prior to testing for an association between SNPs and TG levels, the TG level was logarithmically transformed and adjusted for gender, age, BMI, hypertension, physical activity, smoking, and alcohol consumption by using a multiple linear regression analysis. Then, the adjusted log-transformed TG level was used as the trait in subsequent association analyses.

In the SAPPHiRe sample, the association of each tag-SNP with TG levels was examined by implementing a simple linear regression analysis, which used the adjusted log-transformed TG level as dependent variable and the SNP genotype code as the independent variable. Subsequently, for any two associated tag-SNPs, a multiple linear regression model using the adjusted log-transformed TG level as dependent variable and genotype codes of these two SNPs as the independent variables was implemented to investigate whether they are independently associated with TG levels. Then, significant associations observed in the SAPPHiRe sample were examined in the TWB sample using a simple linear regression analysis. Furthermore, a chi-squared test was used to examine the difference in genotype distributions of specified SNPs between different sample sets.

In the SAPPHiRe sample, the generalized estimating equation (GEE) approach was implemented for all above-described simple and multiple linear regression analyses to deal with the correlation between subjects from the same families. A significance level of 0.05 was used throughout the statistical testing. When testing for the associations of individual tag-SNPs with TG levels in the SAPPHiRe sample under different genetic models, a false discovery rate (FDR)-based measure of significance,  $q$  value, was calculated by QVALUE software [34] to deal with multiple comparisons. An association with a  $q$  value less than 0.05 was considered significant under multiple comparisons. All statistical analyses were implemented using R software.

## Results

Initially, 979 SAPHIRE Taiwanese participants were genotyped on the *IGF1* SNPs. After excluding subjects using medications for hyperlipidemia and subjects with a TG level of > 7.345 mmol/L (650 mg/dl) [30], 954 siblings from 397 families were used in the current study. On the other hand, according to the sample preprocessing procedure for TWB sample described in "Materials & Methods", 13,193 unrelated TWB subjects were collected and analyzed in this study. Clinical characteristics of the SAPHIRE and TWB samples are summarized in Table 1.

Nine SNPs were identified and genotyped in the SAPHIRE sample, and the relevant information is summarized in Supplementary Table S1. Among the 979 subjects genotyped, proportions of successful genotyping of individual SNPs ranged from 95.0% to 99.8%. Based on these genotypes, the levels of pairwise LD among these nine SNPs were estimated

and are presented in Supplementary Figure S1. According to the threshold of  $r^2 = 0.8$ , five SNPs were selected as tags and used in subsequent association analyses. These five tag-SNPs were rs2288377, rs978458, rs6217, rs6214, and rs6219.

TG levels of the SAPHIRE subjects stratified by the genotype of each tag-SNP are summarized in Table 2. For each tag-SNP, the association with TG levels was tested under additive, dominant, and recessive genetic models. As shown in Table 2, associations were observed in recessive model, but not in additive and dominant models. Specifically, significant associations were revealed by rs2288377 ( $\beta = -0.063$ ,  $p = 0.0072$ ), rs978458 ( $\beta = -0.049$ ,  $p = 0.0043$ ), and rs6217 ( $\beta = -0.055$ ,  $p = 0.047$ ). However, only rs2288377 and rs978458 had  $q$  values less than 0.05. According to these results, associations between *IGF1* SNPs and TG levels were tested under recessive model in subsequent analyses.

**Table 1.** Clinical characteristics of the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPHIRE) and Taiwan biobank (TWB) samples

Variable (units)	SAPHIRE sample		TWB sample	
	<i>n</i>	Mean $\pm$ SD/geometric mean [IQR]/percentage	<i>n</i>	Mean $\pm$ SD/geometric mean [IQR]/percentage
Age (years)	954	48.97 $\pm$ 8.25	13193	48.23 $\pm$ 10.91
BMI (kg/m <sup>2</sup> )	954	25.3 $\pm$ 3.36	13193	24.23 $\pm$ 3.61
Systolic blood pressure (mm Hg)	954	129.78 $\pm$ 26.66	13193	115.64 $\pm$ 17.01
Diastolic blood pressure (mm Hg)	954	77.44 $\pm$ 14.62	13193	72.34 $\pm$ 11.05
Triglyceride (mmol/L)	954	1.24 [0.88, 1.78]	13193	1.09 [0.75, 1.53]
Male (%)	954	47.3%	13193	49%
Medication for hypertension (%)	954	57.8%	-	-
Self-reported hypertension (%)	-	-	13102	10.1%
Hypertension (%)	954	66.9%	13108	18.5%
Family history of hypertension (%)	954	63.8%	13193	21.2%
Current/ever smoker (%)	948	26.9%	13191	31.9%
Current/ever alcohol drinker (%)	943	22.3%	13192	10.6%
Physical activity <sup>a</sup> (%)	952	33.9%	13189	40%

*n*, number of subjects with available data; SD, standard deviation; IQR, interquartile range.

<sup>a</sup> For the SAPHIRE sample, indicates non-sedentary subjects; for the TWB sample, indicates subjects with the exercise habit.

**Table 2.** Association of *IGF1* tag single-nucleotide polymorphisms (SNPs) with triglyceride levels under different genetic models in the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPHIRE) sample

SNP	Genotype ( <i>n</i> )	Triglyceride level Geometric mean [IQR]	Additive model		Dominant model		Recessive model	
			$\beta$ (95% CI) <sup>a</sup>	<i>p</i> ( <i>q</i> )	$\beta$ (95% CI) <sup>a</sup>	<i>p</i> ( <i>q</i> )	$\beta$ (95% CI) <sup>a</sup>	<i>p</i> ( <i>q</i> )
rs2288377	TT (459)	1.25 [0.86, 1.78]	-0.017	0.10	-0.0085	0.54	-0.063	<b>0.0072</b>
	TA (384)	1.26 [0.88, 1.81]	(-0.038, 0.0034)	(0.19)	(-0.036, 0.019)	(0.46)	(-0.11, -0.017)	<b>(0.040)</b>
	AA (88)	1.13 [0.88, 1.53]						
rs978458	GG (283)	1.23 [0.88, 1.74]	-0.016	0.094	-0.0007	0.96	-0.049	<b>0.0043</b>
	AG (456)	1.30 [0.88, 1.91]	(-0.035, 0.0027)	(0.19)	(-0.029, 0.027)	(0.72)	(-0.083, -0.015)	<b>(0.040)</b>
	AA (178)	1.12 [0.80, 1.50]						
rs6217	TT (469)	1.26 [0.86, 1.79]	-0.021	0.056	-0.019	0.16	-0.055	<b>0.047</b>
	TG (386)	1.24 [0.88, 1.77]	(-0.043, 0.00051)	(0.16)	(-0.046, 0.0077)	(0.26)	(-0.11, -0.00076)	(0.16)
	GG (58)	1.13 [0.86, 1.55]						
rs6214	GG (256)	1.28 [0.85, 1.89]	-0.011	0.25	-0.013	0.39	-0.016	0.31
	GA (480)	1.23 [0.88, 1.74]	(-0.030, 0.0079)	(0.35)	(-0.044, 0.017)	(0.44)	(-0.048, 0.015)	<b>(0.39)</b>
	AA (188)	1.23 [0.89, 1.73]						
rs6219	GG (646)	1.26 [0.89, 1.78]	-0.0044	0.75	-0.010	0.53	0.035	0.47
	GA (265)	1.21 [0.78, 1.75]	(-0.032, 0.023)	(0.60)	(-0.040, 0.020)	(0.46)	(-0.060, 0.13)	<b>(0.46)</b>
	AA (24)	1.25 [0.81, 1.63]						

<sup>a</sup> The association was adjusted for gender, age, body mass index, hypertension, physical activity, smoking, and alcohol consumption. IQR, interquartile range; CI, confidence interval.

To examine whether rs2288377 and rs978458 are independently associated with TG levels, an analysis of a two-SNP model was performed. As shown in Table 3, only the association of rs978458 remained significant ( $\beta = -0.040$ ,  $p = 0.032$ ) in the two-SNP model, which suggested that the association of rs2288377 could be explained by the association of rs978458. Therefore, only the association between rs2288377 and TG levels was re-examined in the TWB sample.

**Table 3.** Association of rs2288377 and rs978458 with triglyceride levels under one- and two-single-nucleotide polymorphism (SNP) models in the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRE) sample

Model	rs2288377		rs978458	
	$\beta$ (95% CI)	<i>p</i>	$\beta$ (95% CI)	<i>p</i>
One-SNP model: rs2288377	-0.063 (-0.11, -0.017)	<b>0.0072</b>	-	-
One-SNP model: rs978458	-	-	-0.049 (-0.083, -0.015)	<b>0.0043</b>
Two-SNP model: rs2288377 and rs978458	-0.042 (-0.092, 0.0082)	0.10	-0.040 (-0.076, -0.0034)	<b>0.032</b>

CI, confidence interval.

In the beginning of the replication analysis, the relationship between rs978458 and TG levels was examined based on all TWB subjects, but no significant association was observed (Table 4,  $p = 0.12$ ). Since the proportions of hypertension and of having an FH of hypertension in the SAPPHIRE sample were much higher than those in the TWB

sample (Table 1), the replication analysis was also conducted on TWB subjects stratified by these two characteristics. When TWB subjects were classified into sets of hypertensive and non-hypertensive subjects, the association was significant in hypertensive subjects ( $\beta = -0.027$ ,  $p = 0.011$ ), but not in non-hypertensive subjects ( $p = 0.56$ ). On the other hand, when TWB subjects were stratified by an FH of hypertension, the association was significant in subjects with an FH ( $\beta = -0.045$ ,  $p = 0.000034$ ), but not in subjects without an FH ( $p = 0.61$ ). It is clear that the significant association observed in subjects with an FH was much stronger than that observed in hypertensive subjects.

In addition to considering stratification by the hypertension status and FH of hypertension separately, the association between rs978458 and TG levels was further examined under stratification by both characteristics simultaneously. As shown in Table 5, rs978458 was significantly associated with TG levels in subjects with an FH of hypertension, whether the subjects were hypertensive ( $\beta = -0.039$ ,  $p = 0.0042$ ) or not ( $\beta = -0.051$ ,  $p = 0.00017$ ). Conversely, the association disappeared in subjects without an FH of hypertension, regardless of whether the subjects were hypertensive ( $p = 0.67$ ) or not ( $p = 0.50$ ). Furthermore, no significant difference in the distributions of rs978458 genotypes was observed among these four strata (Supplementary Table S2,  $p = 0.22$ ).

**Table 4.** Association between rs978458 and triglyceride levels under a recessive genetic model in different strata of the Taiwan biobank (TWB) sample

Sample stratum	Genotype ( <i>n</i> )	Triglyceride level Geometric mean [IQR]	Association under a recessive model	
			$\beta$ (95% CI)	<i>p</i>
All subjects	GG/AG (10306)	1.09 [0.75, 1.53]	-0.0069 (-0.015, 0.0017)	0.12
	AA (2769)	1.07 [0.73, 1.49]		
Hypertensive subjects	GG/AG (1936)	1.34 [0.94, 1.86]	-0.027 (-0.048, -0.0063)	<b>0.011</b>
	AA (483)	1.28 [0.92, 1.72]		
Non-hypertensive subjects	GG/AG (8370)	1.04 [0.71, 1.44]	-0.0028 (-0.012, 0.0065)	0.56
	AA (2286)	1.03 [0.70, 1.41]		
Subjects with a family history of hypertension	GG/AG (2241)	1.21 [0.82, 1.70]	-0.045 (-0.064, -0.026)	<b>0.000034</b>
	AA (554)	1.09 [0.80, 1.51]		
Subjects without a family history of hypertension	GG/AG (8065)	1.06 [0.72, 1.48]	0.0025 (-0.0071, 0.012)	0.61
	AA (2215)	1.07 [0.72, 1.47]		

IQR, interquartile range; CI, confidence interval.

**Table 5.** Association between rs978458 and triglyceride levels in Taiwan biobank (TWB) subjects stratified by the hypertension status and family history of hypertension simultaneously

Hypertension status	Subjects with a family history of hypertension				Subjects without a family history of hypertension			
	Genotype	TG level geometric mean [IQR]	$\beta$ (95% CI)	<i>p</i>	Genotype	TG level geometric mean [IQR]	$\beta$ (95% CI)	<i>p</i>
Hypertensive subjects	GG/AG	1.35 [0.95, 1.86]	-0.039 (-0.066, -0.012)	<b>0.0042</b>	GG/AG	1.34 [0.92, 1.85]	-0.0074 (-0.041, 0.026)	0.67
	AA	1.24 [0.88, 1.68]			AA	1.34 [0.96, 1.80]		
Non-hypertensive subjects	GG/AG	1.05 [0.75, 1.41]	-0.051 (-0.077, -0.025)	<b>0.00017</b>	GG/AG	1.04 [0.71, 1.44]	0.0034 (-0.0065, 0.013)	0.50
	AA	0.94 [0.68, 1.30]			AA	1.05 [0.71, 1.44]		

IQR, interquartile range; CI, confidence interval.

**Table 6.** Association between rs978458 and triglyceride levels in the Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRE) subjects stratified by a family history of hypertension

Sample stratum	Genotype (n)	Triglyceride level Geometric mean [IQR]	Association under a recessive model	
			$\beta$ (95% CI)	<i>p</i>
Subjects with a family history of hypertension	GG/AG (474)	1.30 [0.92, 1.87]	-0.068	0.0025
	AA (106)	1.12 [0.80, 1.58]	(-0.11, -0.024)	
Subjects without a family history of hypertension	GG/AG (265)	1.21 [0.80, 1.76]	-0.026	0.32
	AA (72)	1.12 [0.86, 1.43]	(-0.078, 0.025)	

IQR, interquartile range; CI, confidence interval.

According to observations obtained from the TWB sample, a re-examination of the association between rs978458 and TG levels in the SAPPPIRe sample was conducted, in which subjects were stratified by an FH of hypertension. As shown in Table 6, the association was significant in subjects with an FH ( $\beta = -0.068$ ,  $p = 0.0025$ ), but not in subjects without an FH ( $p = 0.32$ ). These results are concordant with those shown in Table 4. In addition, among the subjects having an FH of hypertension, the reduction of geometric mean of TG levels in subjects with AA genotype on rs978458, compared with other subjects, was 9.9% and 13.8% in TWB and SAPPPIRe, respectively (Table 4 and 6).

## Discussion

The *IGF1* gene is located on chromosome 12q23-q24, and this chromosomal region has been linked to TG levels by previous linkage studies [21, 23]. Specifically, based on Caucasian families from the HERITAGE Family Study, Feitosa et al. (2006) obtained a maximum genome-wide LOD score of 2.07 ( $p = 0.00108$ ) for TG levels and reported that this maximum LOD peak coincided with a marker in *IGF1* [23]. In addition, based on Chinese families from the SAPPPIRe study, Hsiao et al. (2006) also obtained a modest peak for TG levels in this region (LOD = 1.30, empirical  $p = 0.0052$ ) [21]. Although *IGF1* levels were associated with TG levels in several studies [25-27], the association between the *IGF1* gene and TG levels was not well investigated and remained unclear.

In this study, based on two independent samples collected by the SAPPPIRe and TWB projects, we found that a common tag-SNP within *IGF1*, rs978458, was significantly associated with TG levels. In addition to providing evidence for an association between rs978458 and TG levels, a novel result of this study is that this association appeared in subjects with an FH of hypertension but not in subjects without such an FH. To the best of our knowledge, this is the largest candidate gene study investigating the relationship between the *IGF1* gene and TG levels to date and the first study providing significant evidence for an association between *IGF1* and TG levels in subjects with an FH of hypertension in an ethnic Chinese (i.e., Taiwanese) population.

Essentially, association analyses performed in this study could be divided into three stages. In the first-stage analysis, based on the entire SAPPPIRe sample, an association was found between rs978458 and TG levels under a recessive genetic model (Table 2 and 3). Specifically, subjects carrying the homozygous genotype of the minor allele on rs978458 had lower TG levels, compared with other subjects. At the beginning of the second-stage analysis, based on the entire TWB sample, we failed to replicate the association observed in the SAPPPIRe sample (Table 4). From Table 1, we observed that the proportions of hypertension and of having an FH of hypertension in the SAPPPIRe sample were much higher than those in the TWB sample. Since FH of hypertension might influence TG levels [35] and TG could be a strong predictor of incident hypertension [36], the absence of association between rs978458 and TG levels in the entire TWB sample might be due to differences in these sample characteristics. Therefore, further stratification analyses were applied to the TWB sample. Subjects were stratified not only by the hypertension status and FH of hypertension separately (Table 4), but also by these two characteristics simultaneously (Table 5). Results from these stratification analyses indicated that the association between rs978458 and TG levels was significant in subjects with an FH but not in the subjects without an FH, regardless of whether the subjects were hypertensive or not. In the final stage, a re-examination of the SAPPPIRe sample confirmed the phenomenon of this association appearing in subjects with an FH but not in subjects without an FH (Table 6). The concordant results obtained from both the SAPPPIRe and TWB samples provided significant evidence that rs978458 is associated with TG levels in the subjects with an FH of hypertension. These findings could improve our knowledge of the genetic determinants of TG levels.

In this study, the successful discovery and replication of the association between rs978458 and TG levels in subjects with an FH of hypertension benefited from the features of the SAPPPIRe and TWB samples. First, according to the study design of the SAPPPIRe project (as described in "Materials & Methods"), the proportion with an FH of

hypertension in the SAPPHiRe sample was much higher than that in the sample taken from the general population in Taiwan, such as the TWB sample (Table 1, Supplementary Table S3). The high proportion of those with an FH of hypertension in the SAPPHiRe sample enabled us to discover the association between rs978458 and TG levels, even though the initial association analysis in the first stage was not restricted to subjects with an FH of hypertension. Second, the enormous size of the TWB sample enabled us to dissect the relationship between rs978458 and TG levels in subgroups of the TWB sample. As shown in Table 5, even in the smallest stratum consisting of hypertensive subjects without an FH, the sample size still reached 862. Based on the large sample size in each stratum, results of the subgroup analysis should be reliable. Furthermore, in addition to showing an example of a genotype-phenotype association appearing in a subpopulation rather than in the entire population, this study demonstrated that such an association could be masked in samples taken from the general population or other subpopulations. Therefore, to conduct successful replication analyses, it is important to pay attention to differences in characteristics between different samples.

On the other hand, some limitations in this study should be noted. First, the two samples used in this study were both collected from Taiwanese population. Since ethnic heterogeneity may exist in genetic determinants of TG levels, it is uncertain whether the observed association would hold in other populations. Therefore, our findings need to be replicated in other populations in the future. Furthermore, although the phenomenon of the association between rs978458 and TG levels appearing in subjects with an FH of hypertension but not in subjects without an FH was successfully demonstrated in both the SAPPHiRe and TWB samples, its cause is unknown. A reasonable explanation for this phenomenon is that TG levels might be related to interactions between the *IGF1* gene and some specific genetic determinants underlying the subpopulation consisting of subjects with an FH of hypertension. To understand the mechanism underlying the association observed in this study, further investigations on genetic features of the subjects with an FH of hypertension and biological pathways related to the *IGF1* gene and TG levels are needed.

In summary, based on the consistent results from two independent samples, this study provided a significant evidence for association between the *IGF1* gene and TG levels in subjects with an FH of hypertension in Taiwan. More efforts are needed to

confirm these findings in other populations and to investigate the role of the *IGF1* gene in regulating TG levels.

## Abbreviations

BMI: body mass index; CI: confidence interval; CVD: cardiovascular disease; DBP: diastolic blood pressure; FDR: false discovery rate; FH: family history; GEE: generalized estimating equation; GWAS: genome-wide association study; HWE: Hardy-Weinberg equilibrium; IGF1: insulin-like growth factor-1; IQR: interquartile range; LD: linkage disequilibrium; MAF: minor allele frequency; SAPPHiRe: the Stanford Asian Pacific Program in Hypertension and Insulin Resistance; SBP: systolic blood pressure; SD: standard deviation; SNP: single-nucleotide polymorphism; TG: triglyceride; TWB: Taiwan biobank.

## Acknowledgements

We thank all subjects who participated in the SAPPHiRe and TWB projects. We are grateful to Mr. Le-Ting Lin and Mr. Ching-Chang Chou for their assistance with computing. We thank all group members of the SAPPHiRe and TWB projects for their help. We would also like to thank National Core Facility for Biopharmaceuticals (NCFB, MOST 106-2319-B-492-002) and National Center for High-performance Computing (NCHC) of National Applied Research Laboratories (NARLabs) of Taiwan for providing computational resources and storage resources. This study was supported in part by the grants from the National Health Research Institutes, Taiwan (PH-104-PP03, PH-105-PP03, PH-106-PP03) and the Ministry of Science and Technology, Taiwan (MOST106-2314-B-038-052-MY3).

## Author Contributions

WCW, TQ, YDIC, and CAH contributed to the study design; YFC, RHC, CMH, IITL, CHL, YCC, and CAH contributed to data acquisition; TQ, YDIC, and CAH contributed to genotyping data; WCW, YFC, RHC, KYH, and CAH performed data processing and statistical analyses; WCW and CAH contributed to the interpretation of data and drafted the manuscript; CAH is responsible for the integrity of the work as a whole. All authors approved the final version for publication.

## Supplementary Material

Supplementary figure and tables.

<http://www.medsci.org/v15p1035s1.pdf>

## Competing Interests

The authors have declared that no competing interest exists.

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