

Bivariate genome-wide scan for metabolic phenotypes in non-diabetic Chinese individuals from the Stanford, Asia and Pacific Program of Hypertension and Insulin Resistance Family Study

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Abstract

Aims/hypothesis Hypertension, obesity, impaired glucose tolerance and dyslipidaemia are metabolic abnormalities that often cluster together more often than expected by chance alone. Since these metabolic variables are highly heritable and are at least partially genetically determined, the clustering of defects in these traits implies that pleiotropic effects, where a common set of genes influences more than one trait simultaneously, are likely.

Methods We conducted bivariate linkage analyses for highly correlated traits, aiming to dissect the genetic architecture affecting these traits, in 411 Chinese families participating in the Stanford Asia–Pacific Program of Hypertension and Insulin Resistance Study.

Results We confirmed the pleiotropic effects of the locus at 37 cM on chromosome 20 on the following pairs: (1) fasting insulin and insulin AUC (empirical $p=0.0006$); (2) fasting insulin and homeostasis model assessment of

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beta cell function (HOMA-beta) (empirical $p=0.0051$); and (3) HOMA of insulin resistance (IR) and HOMA-beta (empirical $p=0.0044$). In addition, the peak logarithm of the odds (LOD) scores of linkage between a chromosomal locus and a trait for the pair fasting insulin and HOMA-IR rose to 5.10 (equivalent LOD score in univariate analysis, $\text{LOD}_{[1]}=4.01$, empirical $p=8.0\times 10^{-5}$) from 3.67 and 3.42 respectively for these two traits in univariate analysis. Additional significant linkage evidence, not shown in single-trait analysis, was identified at 45 cM on chromosome 16 for the pair 1 h insulin and the AUC for insulin, with a LOD score of 4.29 (or $\text{LOD}_{[1]}=3.27$, empirical $p=2.0\times 10^{-4}$). This new locus is also likely to harbour the common genes regulating these two traits ($p=1.73\times 10^{-6}$).

Conclusions/interpretation These data help provide a better understanding of the genomic structure underlying the metabolic syndrome.

Keywords Bivariate linkage · Chinese · Co-incident linkage · Genetic pleiotropy · LOD scores · Metabolic syndrome · Quantitative trait locus · QTL · Variance component method

Abbreviations

HOMA-beta	homeostasis model assessment of beta cell function
HOMA-IR	homeostasis model assessment of insulin resistance
LOD	logarithm of the odds
QTL	quantitative trait locus
SAPPHIRE	Stanford, Asia and Pacific Program of Hypertension and Insulin Resistance

Introduction

Metabolic syndrome, also known as the insulin resistance syndrome, a cluster of metabolic disorders including insulin resistance, glucose intolerance, hypertension, dyslipidaemia and obesity, is associated with a high risk of developing cardiovascular diseases and type 2 diabetes, as well as with increased all-cause mortality [1–3]. It is known that components of the metabolic syndrome coexist more often than expected by chance alone [4–6]. The clustering of these metabolic traits along with their heritable features suggests that defects in one or more genes may contribute to metabolic syndrome.

Linkage analysis has been widely adopted to narrow down the broad chromosomal regions harbouring susceptibility loci and to elucidate genetic features of various metabolic phenotypes, as well as to categorically define metabolic syndrome [7–9]. The results, however, are mostly limited to identifying: (1) regions with logarithm of the odds (LOD)

scores of linkage between a chromosomal locus and a trait that are of merely marginal significance; or (2) regions with modest contributions to overall trait variation [10]. Several attempts have been made to increase the statistical power of quantitative trait locus (QTL) detection, including the use of composite metabolic syndrome traits [11] and meta-analysis; however, both approaches suffer from the presence of genetic and environmental heterogeneity [12, 13]. Recently, principal component analysis has been applied to associate different ‘latent’ factors with different grouped metabolic variables, with the latent factors then being used as new phenotypes in the subsequent genome-wide linkage. New interesting loci, not revealed from the original individual phenotypes in linkage analysis, were detected [14–16]. However, the biological nature of the latent factors is not clear.

We have previously shown in the Stanford, Asia and Pacific Program of Hypertension and Insulin Resistance (SAPPHIRE) study that the metabolic phenotypes are inheritable and highly correlated [17, 18]; in addition, we have localised a few susceptibility loci for multiple metabolic phenotypes in the Chinese population [9]. In the present study, we applied bivariate linkage analysis, which could provide improved power over univariate analysis [15, 19], to decipher the genetic architecture of the multiple metabolic phenotypes by examining the pleiotropic effects of the mapped loci for pairwise metabolic traits.

Methods

The SAPPHIRE study The SAPPHIRE study was designed to investigate susceptibility genes for hypertension [17] and insulin resistance in selected Chinese and Japanese populations [17, 20]. The details of recruitment, exclusion criteria, phenotyping and genotyping have been described elsewhere [17, 20, 21]. In brief, the study was composed of concordant siblings (all siblings with hypertension) and discordant siblings (at least one hypertensive sibling). Proband was recruited on the basis of the following: age at onset 35–60 years or age >60 years, but with documentation of hypertension status prior to age 60 available. A total of 2,525 subjects of Japanese or Chinese descent were recruited from centres at San Francisco, Hawaii and Taiwan. All of the subjects underwent a clinical and fasting laboratory examination, with written informed consent obtained prior to examination. Diabetic individuals diagnosed on the basis of the WHO criteria as a result of SAPPHIRE laboratory work or those previously diagnosed were excluded from our analyses, as they usually have abnormal traits measures. Since the number of Japanese in this study was very limited (352 in total) and it’s helpful to study a genetically homogeneous population, we focused on the Chinese population in the previous and present

studies. In addition, 32 subjects taking lipid-lowering medicine were excluded from our analyses. To identify non-paternities, we used ‘sib_kin’, a module of ASPEX (available from <http://aspex.sourceforge.net/>, last accessed in May 2007). When non-paternity was identified, that individual was excluded from the analysis [22]. Altogether, 1,365 non-diabetic Chinese subjects (118 parents and 1,247 siblings) with genotyping data from 411 nuclear families were included in this study. The numbers of families with 1 to 8 siblings are 29, 138, 122, 63, 37, 16, 5 and 1 respectively. The study was approved by the institutional review boards at all participating sites.

Genotyping Details about genotyping procedures were described in our previous study [9]. The genotyping procedure used 376 autosomal markers representing short tandem repeat polymorphisms and yielded an average map density of 10 cM. Genotyping quality was monitored by typing 30 samples in duplicate. An error rate of ~1% was estimated on the basis of these duplicate samples. A published sex-averaged genetic linkage map available from the Marshfield Website (available from <http://research.marshfieldclinic.org/genetics>, last accessed in May 2007) was used for linkage analysis, the marker order has been confirmed based on a recent release from this site, see Ranade et al. [22] for additional details. ASPEX software was used to examine Mendelian inconsistencies. In the situations where an error was found, the marker data were converted to missing; less than 1% of the marker data were converted to missing.

Phenotyping After an 8 to 10 h overnight fast, the participants underwent anthropometric measurements at 08:00 hours without wearing shoes and heavy clothes. Each subject took a 75 g OGTT after the anthropometric measurements. Fasting blood samples were collected for the measurements of plasma glucose, insulin, total cholesterol, triacylglycerol and LDL-, VLDL- and HDL-cholesterol levels. Then, 75 g glucose monohydrate (in 300 ml water) was administered to the subject to drink over 5 min. Blood samples were taken for plasma glucose and insulin 1 and 2 h after glucose loading. The patients were not allowed to eat or drink until the end of the test [21].

Phenotyping procedures for plasma glucose and insulin were as detailed in Ranade et al. [21]. Derivations from the homeostasis model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA-beta), AUC glucose and AUC insulin were identical to the previous study [9]. Log transformations or Box–Cox transformations were applied to make the conditional distributions (conditional on the covariates) of the phenotypes more near-normal, as in the previous study [9]. The coefficients of kurtosis for all the traits are less than 2, which appeared not to induce grossly inflated type I error [23].

Covariates We adjusted for the same set of covariates which were adjusted for in the univariate analysis reported previously [9], including BMI, smoking, physical activity, alcohol consumption, field centre and hypertension medication. BMI was calculated as weight in kilograms divided by the square of height in metres (kg/m^2). Smoking was classified by four categories: non-smokers, ex-smokers, current light smoker (at least 1, but less than 15 cigarettes per day) and current heavy smoker (at least 15 cigarettes per day). Physical activity was an indicator variable categorising whether the proportion of hours spent on sedentary activity to daily non-basal activities (calculated as 24 h minus hours of basal activity, including sleeping or lying down) was greater than 0.5 or not. Alcohol consumption was categorised as non-drinker, light drinker, moderate drinker and heavy drinker according to daily average consumption in ounces of alcohol [21]. The recruitment centres included Taiwan, San Francisco and Hawaii. The hypertensive medications possibly affecting plasma and glucose levels [24] included ACE inhibitors and angiotensin receptor blockades. To adjust for these factors, indicator variables were created for all the categorical covariates. Two indicator variables were created to indicate the effects from taking ACE inhibitors and angiotensin receptor blockades, compared with those from not taking these two medications, for the phenotypes related to plasma and glucose levels. Two indicator variables were created for the San Francisco and Hawaii centres respectively, their effects were assessed relatively to the Taiwan centre (baseline). The baseline categories for physical activities, smoker and alcohol consumption were non-basal activities, non-smokers, non-drinking respectively. These covariates were considered to be possible confounders and were all included in these analyses regardless of their significances.

Genome-wide bivariate multipoint linkage analyses The univariate variance component method [25] decomposes the variability of a trait into components for a QTL, the residual polygenic component and the random environmental component [26], which has been extended to a multivariate framework [27]. In the bivariate model, the additive genetic (ρ_g) and environmental (ρ_e) correlations between the two traits represent the effects of shared genes or pleiotropy and shared environmental factors, respectively on the phenotypic variance in a trait. The phenotypic correlations (ρ_p) can be derived from the equation

$$\rho_p = \rho_g \sqrt{h_a^2} \sqrt{h_b^2} + \rho_e \sqrt{1 - h_a^2} \sqrt{1 - h_b^2}$$

where h_i^2 is the heritability for trait i and $i=a, b$, representing either trait 1 or 2. We conducted a series of bivariate quantitative genetic analyses for all pairwise combinations of metabolic traits using the bivariate approach implemented

in SOLAR 2.0 (available from <http://www.sfbr.org/solar>, last accessed in May 2007).

We obtained trait-specific estimates of the variance components due to the QTL, residual genetic factors and environmental factors, respectively, as well as the three associated correlations ρ_q , ρ_g and ρ_e , where ρ_q is a measure of shared major gene effects at the QTL where linkage is being assessed. Under this model, we tested the null hypothesis that the variance component attributed from the QTL equals zero for both traits by comparing the likelihood of this restricted model to that of a model in which this variance component was estimated for the traits. Twice the difference in log likelihoods of these models yields a test statistic that is asymptotically distributed as a mixture of $1/4 \chi_0^2$, $1/2 \chi_1^2$ and $1/4 \chi_3^2$ [27]. Bivariate linkage analyses were performed for each pairwise combination of metabolic traits. LOD scores obtained from bivariate linkage analyses were also converted to a $1/2 \chi_1^2$ of equivalent p values in univariate linkage analyses, hereafter denoted by $\text{LOD}_{[1]}$. To achieve levels of statistical significance comparable to the LOD score thresholds required in the univariate linkage analyses, we considered bivariate multipoint LOD scores ≥ 4.0 to be statistically significant evidence of linkage ($p \leq 0.0001$), ≥ 2.87 as suggestive evidence ($p \leq 0.001$) and ≥ 2.06 as tentative evidence of linkage ($p \leq 0.007$) [28]. However, only regions showing significant or suggestive evidence of linkage are reported here.

To test pleiotropy and co-incident linkage, likelihoods for a linkage model in which ρ_q was estimated were compared with models in which ρ_q was constrained to 0 (no shared major gene effects in the region, i.e. co-incident linkage) and to 1 or -1 (complete pleiotropy) [29]. In the case of ρ_q being constrained to 0, the difference between these likelihoods is distributed as a chi-square with 1 degree of freedom. When ρ_q is constrained to 1 or -1 , a boundary, the difference in likelihoods, is distributed as a $1/2:1/2$ mixture of χ_1^2 and a point mass at 0 [27]. The hypothesis of $\rho_q=1$ or -1 (complete pleiotropy) or the hypothesis of $\rho_q=0$ (co-incident linkage) was rejected when p was less than 0.05.

Results

Descriptive statistics for demographic characters and eighteen metabolic phenotypes used in bivariate analyses are summarised in Table 1. The genetic, environmental and phenotypic correlations (\pm standard errors) between selected pairs of traits are reported in Table 2, with regions showing suggestive or significant evidence. Regions with peak LOD scores ≥ 2.87 (equivalent to an LOD score of 2.0 in univariate analysis) and the results for testing of QTL

Table 1 The clinical and metabolic characteristics of family members in bivariate analyses

Trait	Number of members	Mean \pm SD or %
Female	649	55.9
Age (years)	1,161	50.12 \pm 10.92
Fasting glucose (mmol/l)	1,156	4.93 \pm 0.58
1 h glucose (mmol/l)	1,029	9.34 \pm 2.22
2 h glucose (mmol/l)	1,002	7.20 \pm 1.80
Fasting insulin (pmol/l)	1,153	52.67 \pm 34.97
1 h insulin (pmol/l)	1,023	548.20 \pm 408.14
2 h insulin (pmol/l)	1,021	443.08 \pm 410.09
HOMA-IR	1,152	1.65 \pm 1.21
HOMA-beta	1,152	108.04 \pm 155.72
AUC glucose (mmol/l \times h)	998	16.62 \pm 3.10
AUC insulin (pmol/l \times h)	1,014	796.78 \pm 572.45
Triacylglycerols (mmol/l)	1,148	1.36 \pm 0.84
HDL-cholesterol (mmol/l)	1,152	1.14 \pm 0.33
LDL-cholesterol (mmol/l)	1,134	3.03 \pm 0.94
VLDL-cholesterol (mmol/l)	1,139	0.61 \pm 0.33
Total cholesterol (mmol/l)	1,156	4.77 \pm 0.99
Systolic BP (mmHg)	1,033	127.53 \pm 24.75
Diastolic BP (mmHg)	1,033	76.41 \pm 13.58
BMI (kg/m ²)	887	25.02 \pm 3.27

pleiotropy were performed for these traits and are reported in Tables 3 and 4.

The univariate and bivariate linkage profiles are summarised in Fig. 1. In bivariate linkage analyses, when fasting insulin was paired up with HOMA-IR ($\text{LOD}_{[1]}=4.01$, empirical $p=0.00008$), fasting glucose ($\text{LOD}_{[1]}=3.09$, empirical $p=0.0002$), AUC insulin ($\text{LOD}_{[1]}=2.63$, empirical $p=0.0006$), HOMA-beta ($\text{LOD}_{[1]}=2.81$, empirical $p=0.0051$) or BMI ($\text{LOD}_{[1]}=2.82$, empirical $p=0.00032$), the peak of LOD scores remained located at 37 cM on chromosome 20, the same QTL identified from the univariate analyses for fasting insulin, HOMA-IR and HOMA-beta [9]. This QTL was also found for the trait pairs HOMA-IR/HOMA-beta ($\text{LOD}_{[1]}=2.62$, empirical $p=0.0044$) and HOMA-IR/BMI ($\text{LOD}_{[1]}=2.61$, empirical $p=0.00028$). The complete pleiotropic effects were confirmed for the pairs fasting insulin/AUC insulin, fasting insulin/HOMA-beta and HOMA-IR/HOMA-beta (Table 4), where the hypothesis of co-incident linkage was rejected ($p < 0.05$) and the hypothesis of pleiotropy linkage was not rejected ($p > 0.05$). In contrast, the correlations between these pairs of metabolic variables under a polygenic model are mostly significantly away from zero, except for the pairs BMI/fasting insulin or BMI/HOMA-IR (Table 2). The hypotheses of complete co-incident linkage and pleiotropy were both rejected for the highly correlated trait-pair fasting insulin/HOMA-IR, indicating that the common genetic effect may be modified by environmental influences or the effect of epistasis.

Table 2 Phenotypic (ρ_p), genetic (ρ_g) and environmental (ρ_e) correlations between selected pairs of metabolic phenotypes

Pairs of metabolic traits		ρ_e	ρ_g	ρ_p
Fasting insulin	Fasting glucose	0.41±0.1*	0.21±0.11	0.28±0.06*
Fasting insulin	HOMA-beta	0.83±0.04*	0.49±0.09*	0.66±0.11*
Fasting insulin	HOMA-IR	0.99±0.0023 *	0.96±0.01*	0.98±0.14*
Fasting insulin	AUC insulin	0.55±0.06*	0.66±0.09*	0.59±0.07*
Fasting insulin	BMI	0.14±0.09	-0.2±0.14	0.0021±0.03
Fasting glucose	SBPc	-0.00017±0.11	-0.1±0.14	-0.04±0.03
HOMA-IR	HOMA-beta	0.78±0.06*	0.23±0.12	0.51±0.10*
HOMA-IR	BMI	0.09±0.09	-0.13±0.14	0.0017±0.03
Systolic BP	Diastolic BP	0.75±0.03*	0.51±0.22*	0.7±0.087*
1 h insulin	AUC insulin	0.95±0.01*	0.96±0.02*	0.94±0.14*
1 h insulin	HDL-cholesterol	-0.02±0.1	-0.29±0.12*	-0.15±0.04*
AUC insulin	HDL-cholesterol	-0.05±0.11	-0.3±0.11*	-0.18±0.03*
HDL-cholesterol	VLDL-cholesterol	-0.24±0.11*	-0.44±0.09*	-0.35±0.04*
HDL-cholesterol	Triacylglycerols	-0.27±0.11*	-0.43±0.09*	-0.36±0.05*
HDL-cholesterol	BMI	-0.45±0.12*	0.35±0.11*	-0.01±0.05
2 h insulin	VLDL-cholesterol	0.08±0.1	0.5±0.11*	0.28±0.03*
Total cholesterol	LDL-cholesterol	0.87±0.02*	0.94±0.01*	0.91±0.13*

Values are means±SE

* $p < 0.05$

In the univariate analyses, we did not identify any susceptibility QTL for systolic or diastolic blood pressure; however, the QTL at 88 cM became noticeable with a $LOD_{[1]}$ of 2.46 (empirical $p = 0.00024$) when looking at these two traits simultaneously. A peak at 93 cM in this region also manifested itself when modelling fasting glucose and systolic blood pressure ($LOD_{[1]} = 3.12$, empirical $p = 0.00004$) together. However, this evidence of linkage could be caused by co-incident effects, since the hypothesis of complete co-incident linkage was not rejected ($p > 0.05$) in both regions. Although the environmental and genetic correlations between systolic and diastolic blood pressure are as high as 0.75 ± 0.03 and 0.51 ± 0.22 , respectively, these correlations are almost negligible between systolic blood pressure and fasting glucose. The peaks at around 51 cM on chromosome 20 that we observed in univariate analyses for fasting glucose became more significant when combined with systolic blood pressure ($LOD_{[1]} = 2.06$, empirical $p = 0.0025$) in bivariate analyses.

Several pair-wise combinations with HDL-cholesterol showed similar patterns to HDL-cholesterol with two peaks identified at 113 and 123 cM on chromosome 12 respectively, including the pairs HDL-cholesterol/VLDL-cholesterol, HDL-cholesterol/triacylglycerols, HDL-cholesterol/BMI, HDL-cholesterol/1 h insulin and HDL-cholesterol/AUC insulin. Their $LOD_{[1]}$ scores ranged from 2.15 to 3.01 with empirical p values varying from 0.00012 to 0.0018. These QTLs, however, could have been caused by co-incident effects, as we failed to reject the hypothesis of complete co-incident linkage while the hypothesis of complete pleiotropy was rejected, except for HDL-cholesterol/BMI, HDL-

cholesterol/1 h insulin and HDL-cholesterol/AUC insulin (both hypotheses not rejected; Table 4). The genetic correlations between HDL-cholesterol and these traits are all statistically significant as shown in Table 2. In addition, the linkage signals became more substantial in bivariate analysis than in univariate analysis for the following paired traits: total cholesterol/LDL on chromosome 1p, fasting insulin/fasting glucose on chromosome 2p, fasting insulin/HOMA-beta on chromosome 18q and 14q and HOMA-IR/HOMA-beta on 14q.

A new QTL located at 148 cM on chromosomes 9, but not discovered in the univariate linkage analyses, was identified for the combinations HOMA-IR/HOMA-beta ($LOD_{[1]} = 2.22$, empirical $p = 0.0083$) and fasting insulin/HOMA-beta ($LOD_{[1]} = 2.47$, empirical $p = 0.009$). The peak for 2 h insulin remained in the same region after combining with VLDL-cholesterol ($LOD_{[1]} = 2.02$, empirical $p = 0.001$); however, this could be attributed to a co-incident effect on this QTL ($p = 0.81$). Interestingly, the QTL located at 45 cM for 1 h insulin and AUC insulin on chromosome 16 ($LOD_{[1]} = 3.27$, empirical $p = 0.0002$) but not identified in the univariate linkage analyses also showed significant evidence of pleiotropic effects (Table 4).

In summary, since AUC insulin was derived from three insulin-related measures and HOMA-beta and HOMA-IR were derived from fasting insulin and fasting glucose, these traits are similar in nature. Linkage signals observed at 37 cM on chromosome 20 from the trait-pairs including fasting insulin/HOMA-beta, fasting insulin/AUC insulin and HOMA-IR/HOMA-beta resulted from the effects of pleiotropy. On the other hand, the linkage evidence observed for

Table 3 Regions with peak LOD scores ≥ 2.87 in genome-wide bivariate multipoint linkage analysis

Trait 1	Trait 2	Chromosome	cM	MLS	MLS _[1]	Empirical <i>p</i> value
Fasting insulin	Fasting glucose	2	90	2.87	2.00	0.0028
		20	37	4.09	3.09	0.0002
		20	73	2.89	2.022	0.0027
Fasting insulin	HOMA-beta	9	148	3.40	2.47	0.009
		14	43	3.48	2.54	0.0084
		18	55	3.05	2.16	0.014
		20	37	3.78	2.81	0.0051
Fasting insulin	HOMA-IR	20	21	3.35	2.43	0.0038
		20	28	3.65	2.69	0.0021
		20	37	5.10	4.01	0.00008
Fasting insulin	AUC insulin	5	85	3.18	2.28	0.0016
		20	37	3.58	2.63	0.0006
Fasting insulin	BMI	20	37	3.79	2.82	0.00032
Fasting glucose	Systolic BP	20	51	2.93	2.06	0.00252
		20	54	2.91	2.04	0.0026
		20	93	4.12	3.12	0.00004
HOMA-IR	HOMA-beta	9	148	3.11	2.22	0.0083
		14	44	3.45	2.51	0.0052
		20	37	3.57	2.62	0.0044
HOMA-IR	BMI	20	37	3.56	2.61	0.00028
Systolic BP	Diastolic BP	20	88	3.39	2.46	0.00024
1 h insulin	AUC insulin	16	45	4.29	3.27	0.0002
1 h insulin	HDL-cholesterol	12	114	3.06	2.17	0.0018
AUC insulin	HDL-cholesterol	12	113	3.03	2.15	0.0014
HDL-cholesterol	VLDL-cholesterol	12	113	4.01	3.01	0.00012
		12	123	3.29	2.38	0.00076
HDL-cholesterol	Triacylglycerols	12	113	3.72	2.76	0.0004
		12	123	3.07	2.18	0.0011
HDL-cholesterol	BMI	12	113	3.75	2.78	0.00052
		12	124	3.57	2.62	0.00064
Adiponectin	HDL-cholesterol	12	115	3.12	2.22	0.00068
		12	126	3.29	2.37	0.00052
		15	31	3.71	2.74	0.00028
2 h insulin	VLDL-cholesterol	9	77	2.89	2.02	0.001
Total cholesterol	LDL-cholesterol	1	71	3.30	2.38	0.00096

BP Blood pressure, *MLS* maximum LOD score in bivariate linkage analysis, *MLS_[1]* the bivariate LOD score converted to LOD score with equivalent *p* value in univariate linkage analyses

most of the trait-pairs comprised of different metabolic measures, such as fasting glucose/systolic blood pressure (on 20q13.32), systolic/diastolic blood pressure (on 20q13.31), HDL-cholesterol/VLDL-cholesterol (on 12q21.33-12q22), HDL-cholesterol/triacylglycerols (on 12q21.33-12q22) and 2 h insulin/VLDL-cholesterol (on 9q13-9q21.1) was mostly due to co-incident effects. BMI doesn't seem to be correlated with fasting insulin or HOMAIR in our study, but it is genetically and environmentally correlated with HDL-cholesterol, with the genetic and environment correction coefficients 'pointing' in the opposite directions. The genetic correlation between BMI and HDL-cholesterol is probably due to the pleiotropy effect from the QTL on chromosome 12.

Discussion

In this study, we present evidence that bivariate linkage analyses of inter-related metabolic phenotypes improve the ability to localise susceptibility loci for one trait. We also show that bivariate analyses appear to be able to differentiate between, on the one hand, pleiotropic effects of a single locus influencing all the traits and, on the other hand, separate tightly clustered loci each influencing a single trait, thus facilitating our understanding of genomic structure for the complex nature of metabolic syndrome.

By applying the bivariate linkage approach, we identified a few putative QTLs for multiple inter-related metabolic phenotypes that are clustered on chromosomes

Table 4 Pleiotropy and co-incident linkage at QTLs identified from the genome-wide bivariate linkage analyses

Bivariate	Chromosome	cM	MLS	<i>p</i> value of the test			
				Co-incident linkage		Pleiotropy	
				$\rho_q=0$	$\rho_q=1$	$\rho_q=-1$	
Fasting insulin	Fasting glucose	20	37	4.09	0.31	0.095	
Fasting insulin	HOMA-beta	20	37	3.78	0.0018 ^a	0.16 ^a	
Fasting insulin	HOMA-IR	20	37	5.10	6.71×10^{-5}	0.0058	
Fasting insulin	AUC insulin	20	37	3.58	1.52×10^{-4a}	0.5 ^a	
Fasting insulin	BMI	20	37	3.79	0.57	0.0025	0.50
Fasting glucose	SBP	20	93	4.12	0.38 ^b	3.61×10^{-4}	0.0095 ^b
HOMA-IR	HOMA-beta	20	37	3.57	0.0030 ^a	0.14 ^a	
HOMA-IR	BMI	20	37	3.56	0.54	0.27	0.50
SBP	DBP	20	88	3.39	0.086 ^b	0.0055 ^b	
1 h insulin	AUC insulin	16	45	4.29	1.73×10^{-6}	2.04×10^{-7}	0.5
1 h insulin	HDL-cholesterol	12	114	3.06	0.36 ^b	0.036 ^b	0.39
AUC insulin	HDL-cholesterol	12	113	3.03	0.32 ^b	0.0036 ^b	0.50
HDL-cholesterol	VLDL-cholesterol	12	113	4.01	0.15 ^b	0.0058	0.012 ^b
HDL-cholesterol	Triacylglycerols	12	113	3.72	0.15 ^b	0.0092	0.020 ^b
HDL-cholesterol	BMI	12	113	3.75	0.54 ^b	0.15	0.0041 ^b
2 h insulin	VLDL-cholesterol	9	77	2.89	0.81 ^b	0.031 ^b	
Total cholesterol	LDL-cholesterol	1	71	3.30	0.0022	0.015	

DBP Diastolic blood pressure, MLS maximum LOD score, SBP systolic blood pressure

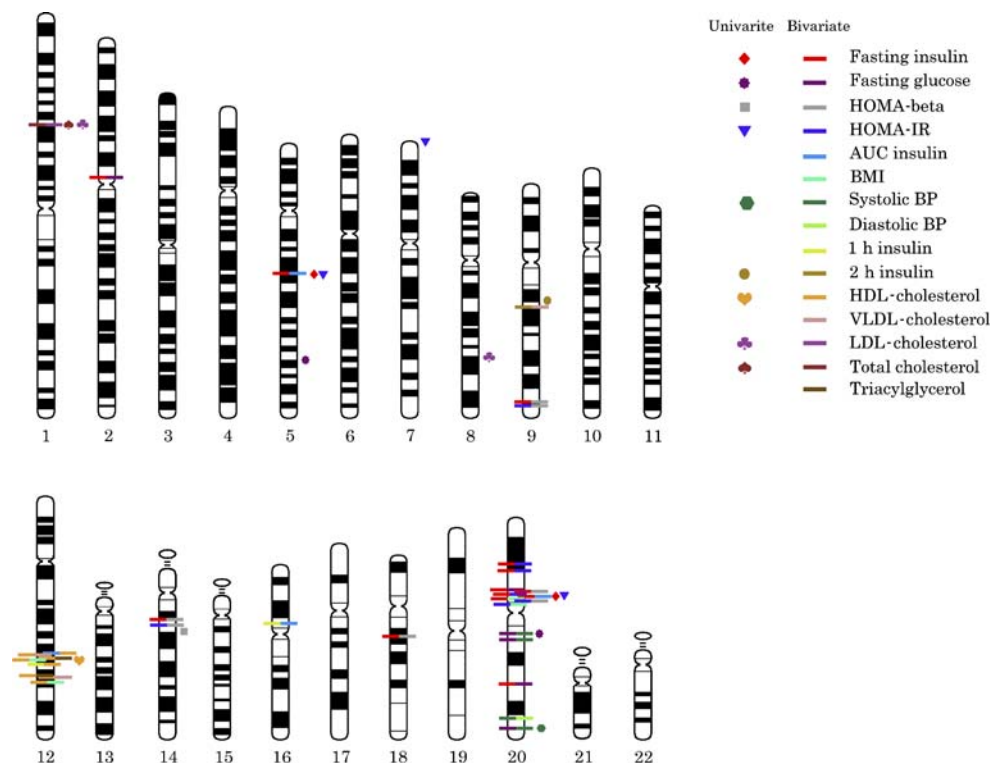
^a Evidence of pleiotropy linkage

^b Evidence of co-incident linkage

12 and 20. Additionally, we found significant or suggestive evidence of linkage of 10 QTLs for various metabolic variables scattered on chromosomes 1p, 2p, 5q, 9q, 14q, 16p and 18q. In general, the LOD scores of these loci were

higher than those obtained in univariate linkage analyses, thus substantiating the results showing only weak evidence of linkage in the previous report [9]. We also found new QTLs on chromosome 9 (LOD_[1]=2.47 and 2.22 at 148 cM

Fig. 1 Total genome review of quantitative trait univariate and bivariate linkage analyses. Results with peak LOD scores ≥ 2.0 are displayed (LOD scores from bivariate linkage analysis have been converted to LOD scores with equivalent *p* values in univariate analysis)



for the trait pairs fasting insulin/HOMA-beta and HOMA-IR/HOMA-beta, respectively) and on chromosome 16 (LOD_[1]=3.27 at 45 cM for 1 h insulin/AUC insulin) that have not been identified in the univariate linkage analyses. There are roughly 35 QTLs showing only tentative evidence of linkage with LOD_[1] ≥ 2.00, indicating that bivariate linkage analysis is a more powerful approach than univariate analysis.

The QTL at 71 cM on chromosome 1 identified for total cholesterol (LOD=2.16) in the univariate analysis became slightly more substantial for the trait pair total cholesterol/LDL cholesterol (LOD_[1]=2.38). The susceptibility QTL for fasting insulin, HOMA-IR and HOMA-beta located at 37 cM on chromosome 20, a region previously identified, was also identified in this study, with LOD scores of 3.78 to 5.10 (or LOD_[1] scores of 2.81–4.01) compared with LODs of 1.71 to 3.65 as estimated in the univariate analysis [9]. Interestingly, in the bivariate analyses, this locus also influenced fasting glucose, AUC insulin and BMI. Although the genetic correlations between fasting insulin and fasting glucose or BMI are relatively low, possible genetic pleiotropy for these metabolic phenotypes was observed. This notion is particularly true for fasting insulin and HOMA-beta or AUC insulin, which are consistent with a hypothesis of complete pleiotropy. As for the interaction between fasting insulin and glucose levels, we cannot exclude the possibility of partial pleiotropy. These findings are consistent with previous studies showing that trait pairs exhibiting low to moderate overall genetic and phenotypic correlations appear more informative for bivariate analyses than pairs with high genetic correlations [19, 27, 30, 31]. On the other hand, the QTL responsible for fasting insulin together with BMI or HOMA-IR could be due to co-incident linkage, suggesting that the susceptibility genes are independently responsible for fasting insulin or BMI and simply happen to cluster together.

The candidate gene proprotein convertase subtilisin/kexin type 2 (*PCSK2*), which is located at the region on chromosome 20, has been reported to be associated with type 2 diabetes in a Japanese study [32]. In addition, it is very likely that other genes nearby also contribute to insulin resistance and/or beta cell functions. We are currently working on the identification of SNPs from other candidate genes in this region.

Surprisingly, the two QTLs that peaked at 113 and 123 cM on chromosome 12 for HDL-cholesterol, VLDL-cholesterol and triacylglycerols also showed great influences on 1 h insulin, AUC insulin and BMI. This QTL was also previously identified by univariate linkage analyses [33]. Since the highest peak loci for different trait pairs were all located at 113 cM from pter, this indicates the possibility that the same gene in this region contributes to different phenotypes. However, bivariate linkage analyses

showed that the QTL for the trait pairs HDL-cholesterol/VLDL-cholesterol and HDL-cholesterol/triacylglycerols could be caused by co-incident linkage. The complete co-incident linkage or pleiotropy hypotheses were not rejected for HDL-cholesterol and BMI. BMI has been shown to have pleiotropic effects with several phenotypes related to insulin resistance syndrome on chromosome 6q [34], yet the phenomenon was not observed in this study. Although we are not clear about the underlying genetic mechanism, the presence of incomplete pleiotropy could reduce the power to reject co-incident linkage. It is likely that several allelic variants of a major putative gene exert a differential influence on the two correlated phenotypes. Or one genetic effect, modified by environmental factors or by genetic epistasis, influences the two correlated phenotypes. Several candidate genes have been reported in the region identified for HDL-cholesterol on chromosome 12, including sterol *O*-acyltransferase 2 (*SOAT2*) on 12q13.13, apolipoprotein F (*APOF*) on 12q13.2, nuclear receptor subfamily 1, group H, member 4 (*NR1H4*) on 12q23.1, transcription factor 1 (*TCF1*) on 12q24 and scavenger receptor class B, member 1 (*SCARB1*) on 12q24.31 [35]. When HDL-cholesterol was paired up with either VLDL-cholesterol, triacylglycerols or BMI in the bivariate analysis, the peak was located at 12q23.2, where the marker PAH closest to the peak has been shown to be a putative QTL with a LOD of 2.13 for HDL-cholesterol levels in the Mexican American population in the San Antonio Family Heart Study [36]. In this region, the *NR1H4* gene, also known as the *FXR* gene, is a key regulator of cholesterol homeostasis, as shown in *Fxr*^{-/-} knockout mice [37].

In search for the genetic loci for metabolic syndrome (as a categorical diagnosis) and its related traits, many studies have been conducted in different populations [11, 14, 16, 38, 39], including Chinese living in Hong Kong [7]. We were unable to replicate the results from that study by again showing evidence of linkage for metabolic syndrome on chromosome 1 at 169.5–181.5 cM and on chromosome 2 at 44.1–57.3 cM. However, we did observe a significant QTL on chromosome 16 at 45 cM (LOD=4.29 and LOD_[1]=3.27 for 1 h insulin and AUC insulin), similar to the reported region at 45.2–65.4 cM (with LOD=1.75, 1.61 and 1.25 for metabolic syndrome, HOMA-IR and HDL-cholesterol respectively) in Hong Kong Chinese [7].

In conclusion, several metabolic phenotypes related to the complex metabolic syndrome might be attributed to common genetic influences, i.e. genetic pleiotropy, as we have observed for obesity/insulin (37 cM on chromosome 20) and lipid/insulin (113 cM on chromosome 12) phenotypes. Interestingly, the genes responsible for fasting insulin, HOMA-beta and AUC insulin might be identical and are located at 37 cM from pter on chromo-

some 20. These data help provide a better understanding of the genetic factors in the chromosomal regions influencing different metabolic traits, thus facilitating the characterisation of the positional candidates that are involved in the pathogenesis of metabolic syndrome in Chinese populations.

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