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Meta-analysis of Gene-Environment interaction: joint estimation of SNP and SNP×Environment regression coefficients

Alisa K. Manning¹, Michael LaValley¹, Ching-Ti Liu¹, Ken Rice², Ping An³, Yongmei Liu⁴, Iva Miljkovic⁵, Laura Rasmussen-Torvik⁶, Tamara B. Harris⁷, Michael A. Province³, Ingrid B. Borecki³, Jose C. Florez^{8,9,10,11}, James B. Meigs^{11,12}, L. Adrienne Cupples^{1,13}, and Josée Dupuis^{1,13}

¹Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA ²Department of Biostatistics, University of Washington, Seattle ³Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, Missouri ⁴Department of Epidemiology and Prevention, Wake Forest University School of Medicine, Winston-Salem, North Carolina ⁵Center for Aging and Population Health, Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA ⁶Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL ⁷Geriatric Epidemiology Section, Laboratory of Epidemiology, Demography, and Biometry, National Institute on Aging, Bethesda, Maryland ⁸Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts ⁹Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts ¹⁰Diabetes Research Center, Diabetes Unit, Massachusetts General Hospital, Boston, Massachusetts ¹¹Department of Medicine, Harvard Medical School, Boston, Massachusetts ¹²General Medicine Division, Massachusetts General Hospital, Boston, Massachusetts ¹³National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, Massachusetts

Abstract

Introduction—Genetic discoveries are validated through the meta-analysis of genome-wide association scans in large international consortia. Because environmental variables may interact with genetic factors, investigation of differing genetic effects for distinct levels of an environmental exposure in these large consortia may yield additional susceptibility loci undetected by main effects analysis. We describe a method of joint meta-analysis of SNP and SNP by Environment (SNP×E) regression coefficients for use in gene-environment interaction studies.

Methods—In testing SNP×E interactions, one approach uses a two degree of freedom test to identify genetic variants that influence the trait of interest. This approach detects both main and interaction effects between the trait and the SNP. We propose a method to jointly meta-analyze the SNP and SNP×E coefficients using multivariate generalized least squares. This approach provides confidence intervals of the two estimates, a joint significance test for SNP and SNP×E terms, and a test of homogeneity across samples.

Results—We present a simulation study comparing this method to four other methods of meta-analysis and demonstrate that the joint meta-analysis performs better than the others when both main and interaction effects are present. Additionally, we implemented our methods in a meta-analysis of the association between SNPs from the type 2 diabetes-associated gene *PPARG* and log-transformed fasting insulin levels and interaction by body mass index in a combined sample of 19,466 individuals from 5 cohorts.

Keywords

2 degree of freedom meta-analysis; joint meta-analysis; PPARG; Gene-environment interaction meta-analysis

Introduction

Genome-wide association studies have facilitated discovery of associations between genetic variants and complex phenotypes. After discovery and replication of the strongest association signals, weaker associations are discoverable only with the increased power of larger samples [McCarthy 2008]. Therefore, individual research groups have formed consortia and meta-analyzed their GWAS of complex diseases to find such signals [Ioannidis 2007, Lindgren 2009, Prokopenko 2009, Psaty 2009]. For commonly used analyses, this approach is as efficient as combining individual participant data [Lin and Zeng 2010]. However, the SNP associations identified by these consortia explain little heritability in the quantitative traits being studied. Many quantitative traits are influenced by environmental factors, which may interact with the genetic loci being tested. As a way to incorporate environmental factors, identify other genetic contributors to the trait of interest, explain the remaining heritability, and to further characterize the genetic architecture of the trait, we now wish to look beyond SNP main effect associations to SNP-environment interaction associations.

In this paper, we propose a method to jointly meta-analyze the beta coefficients for a SNP's main effect and its interaction with an environment variable (SNP×E). This method provides meta-analytic estimates of these coefficients, as well as a joint test of significance and a test of heterogeneity. We present a simulation study to compare the joint meta-analysis to four other meta-analytic methods for detecting SNP associations in quantitative traits. Other methods have been proposed in the context of phenotypes tested in case-control designs [Murcray 2008, Chatterjee 2008]; here we focus on a quantitative trait as the outcome.

As an example illustrating our method, the Meta-Analysis of Glycemic and Insulin-related traits Consortium (MAGIC) has recently published the discovery of 16 novel loci associated with fasting glucose but only 2 novel loci associated with fasting insulin [Dupuis 2010]. The low number of SNPs associated with insulin resistance measures, as compared to those associated with insulin secretion indices, remains an intriguing empiric observation in the field. As an illustration, the MAGIC meta-analysis of 21 studies contributing up to 38,238 individuals to the discovery sample failed to detect associations between log-transformed fasting insulin and SNPs near *PPARG*, a known type-2 diabetes gene [Altshuler 2000]. Fasting insulin, a surrogate indicator of insulin resistance, is highly associated with body mass index (BMI), and it has been suggested that BMI interacts with the *PPARG* locus in influencing type 2 diabetes risk [Florez 2007, Ludovico 2007, Tonjes 2006]. Consequently, we explored methods for identifying main effect of SNPs in *PPARG* on levels of log(fasting insulin), and their interactions with BMI, in a meta-analysis of five cohorts comprising 19,466 individuals.

Methods

For all strategies we assume that association results are gathered from k studies where N SNPs are genotyped and the trait Y and environmental factor E are also measured. From these studies, summary statistics are available from single-SNP regressions assessing the association between Y and each SNP. Two regressions will be considered: the main effects

regression, in which the association between the SNP and Y is tested while adjusting for E , and the interaction regression with both SNP and SNP $\times E$ regression terms.

Main Effects Regression Model

The mean model used in linear regression for main effects is

$$E(Y|E, SNP) = \beta_{0i}^M + \beta_{1i}^M E + \beta_{2i}^M SNP.$$

Here, i is the study index and the superscript M indicates coefficients pertinent to main effects. The environmental variable E can be dichotomous or continuous. Typically, the SNP will be coded as the number of a specific allele, e.g. 0, 1, or 2 minor alleles. Additional covariates may be included in this model, such as age and sex.

Interaction Regression Model

A mean model used in linear regression for interaction effects is

$$E(Y|E, SNP) = \beta_{0i}^I + \beta_{1i}^I E + \beta_{2i}^I SNP + \beta_{3i}^I SNP \times E,$$

where the superscript I indicates coefficients pertinent to interactions. Statistical interaction is assessed by testing the null hypothesis $\beta_{3i}^I = 0$.

Joint Meta-Analysis of SNP and SNP $\times E$ (JMA)

We propose to apply the method of Becker and Wu [2007] to simultaneously summarize the SNP and SNP $\times E$ beta coefficients from fitted interaction models. This method provides a joint test of significance of the estimates and a test of homogeneity of the regression estimates across the k studies. The benefit of the joint meta-analysis is two-fold: first, it can act as a screening tool to find SNPs which may have significant main or interaction effects and second, SNPs may only show significant associations when interaction is considered.

For each study, linear unbiased estimators $\widehat{\beta}_i^I = (\widehat{\beta}_{2i}^I, \widehat{\beta}_{3i}^I)$ and $\widehat{\Sigma}_i^I = \text{cov}(\widehat{\beta}_i^I)$ are computed. We assume the $\widehat{\beta}_i^I$ are asymptotically normally distributed with mean β and variance Σ . The purpose of this method is to estimate β and Σ .

A vector, $\mathbf{b}$, is formed with the $\widehat{\beta}_i^I$ from each cohort. This vector has block-diagonal covariance under the assumption of independence among cohorts:

$$\mathbf{b} = \begin{bmatrix} \widehat{\beta}_{21}^I \\ \widehat{\beta}_{31}^I \\ \widehat{\beta}_{22}^I \\ \widehat{\beta}_{32}^I \\ \vdots \\ \widehat{\beta}_{2k}^I \\ \widehat{\beta}_{3k}^I \end{bmatrix} \text{ and } \widehat{\Sigma} = \begin{bmatrix} \widehat{\Sigma}_1^I & 0 & 0 & 0 \\ 0 & \widehat{\Sigma}_2^I & 0 & 0 \\ 0 & 0 & \ddots & \vdots \\ 0 & 0 & \cdots & \widehat{\Sigma}_k^I \end{bmatrix}.$$

Since each slope vector $\widehat{\beta}_i^T$ estimates β , we can rewrite the vector $\mathbf{b}$ as

$$\mathbf{b} = \mathbf{W}\beta + \mathbf{e} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ \cdots & \cdots \\ \cdots & \cdots \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \beta_2^T \\ \beta_3^T \end{bmatrix} + \mathbf{e},$$

where $\mathbf{e}$ follows a multivariate normal distribution with means 0 and covariance $\widehat{\Sigma}$.

The estimate of β and its covariance are obtained from generalized least squares, accounting for the unequal variances contributed to the estimate from studies of different sizes:

$$\widehat{\beta} = (\mathbf{W}^T \Sigma^{-1} \mathbf{W})^{-1} \mathbf{W}^T \Sigma^{-1} \mathbf{b} \text{ and } \text{Cov}(\widehat{\beta}) = (\mathbf{W}^T \Sigma^{-1} \mathbf{W})^{-1}.$$

Confidence intervals of the beta estimates can be obtained with $\widehat{\beta}_p \pm Z_{1-\alpha/2} \sqrt{C_{pp}}$ where $\widehat{\beta}_p$ is the p^{th} element of $\widehat{\beta}$ and C_{pp} is the p^{th} diagonal element of $\text{Cov}(\widehat{\beta})$. Additionally, $\widehat{\beta}$ and $\text{Cov}(\widehat{\beta})$ can be used to calculate a joint confidence region of β_2^T and β_3^T .

A Wald statistic $T_{JMA} = \widehat{\beta}^T \text{Cov}(\widehat{\beta})^{-1} \widehat{\beta}$ follows a two degree of freedom chi-square distribution under the null hypothesis that $\beta = 0$ and can be used to test the joint significance of $\widehat{\beta}_2^T$ and $\widehat{\beta}_3^T$.

A test of homogeneity of regression coefficients, analogous to the Cochran's Q-test [Cochran 1954, Higgins 2002], can be performed using the statistic $Q = (\mathbf{b} - \mathbf{W}\widehat{\beta})^T \Sigma^{-1} (\mathbf{b} - \mathbf{W}\widehat{\beta})$. Under the null hypothesis that $\widehat{\beta}_i^T = \widehat{\beta}$ for all i , the test statistic $Q = 0$.

The summary beta estimates obtained from the joint meta-analysis are used for the interpretation of the SNP effect for varying levels of the environmental variable. Gathering terms from the model, $E(Y|E, \text{SNP}) = \beta_{0i}^T + \beta_{1i}^T E + \beta_{2i}^T \text{SNP} + \beta_{3i}^T \text{SNP} \times E$, the effect of the SNP can be written:

$$\widehat{\beta}_2^T \text{SNP} + \widehat{\beta}_3^T E \times \text{SNP} = (\widehat{\beta}_2^T + \widehat{\beta}_3^T E) \text{SNP}$$

with corresponding standard error:

$$\text{se}(\widehat{\beta}_2^T + \widehat{\beta}_3^T E) = \sqrt{\text{var}(\widehat{\beta}_2^T) + E^2 \text{var}(\widehat{\beta}_3^T) + 2E \text{cov}(\widehat{\beta}_2^T, \widehat{\beta}_3^T)}.$$

Simulation Study

We designed a simulation study to compare the joint meta-analysis (JMA) strategy to four other meta-analytic methods. The first two produce meta-analyzed summaries of a single term: in the main effects meta-analysis, we summarize the estimate of the SNP term from the main effects regression model and in the interaction meta-analysis, we summarize the estimate of the interaction term from the interaction regression model. The third method,

screening by main effects, restricts the interaction test to the subset of SNPs with main effects p-values lower than a pre-specified threshold α_M . Finally, the fourth method, the meta-analysis of the 2 degree of freedom test, jointly tests the SNP and SNP×E regression coefficients.

Meta-Analysis of SNP Main Effects

In the study of SNP associations, the main effect of a SNP will often be assessed prior to performing interaction regressions. The estimates $\widehat{\beta}_{2i}^M$ can be summarized across the k studies using an inverse-variance weighted meta-analysis [Petitti 2000]. We define

$\bar{\beta}_2^M = \sum_{i=1}^k \frac{w_i \widehat{\beta}_{2i}^M}{\sum_{i=1}^k w_i}$, where $w_i = \frac{1}{\text{var}(\widehat{\beta}_{2i}^M)}$, as a weighted average of the $\widehat{\beta}_{2i}^M$ from each study. Here, the weights are inversely proportional to the estimated variance of the beta estimates.

The estimated standard error of $\bar{\beta}_2^M$ is:

$$\text{se}(\bar{\beta}_2^M) = \sqrt{\frac{1}{\sum_{i=1}^k 1/\text{var}(\widehat{\beta}_{2i}^M)}}.$$

To test the null hypothesis that β_2^M is equal to zero, the test statistic, T_{MAIN} , can be constructed:

$$T_{MAIN} = \left(\frac{\bar{\beta}_2^M}{\text{se}(\bar{\beta}_2^M)} \right)^2,$$

and compared to a one degree of freedom chi-square distribution.

Meta-Analysis of Interaction Effects

The simplest approach to detect possible interaction effects is to summarize the $\widehat{\beta}_{3i}^I$ from the k studies. This method identifies SNPs with significant interaction effects regardless of whether or not there is a main effect of the SNP. We perform the same meta-analysis as

described above with the $\widehat{\beta}_{3i}^I$ obtaining: $\bar{\beta}_3^I = \sum_{i=1}^k \frac{w_i \widehat{\beta}_{3i}^I}{\sum_{i=1}^k w_i}$ with $w_i = \frac{1}{\text{var}(\widehat{\beta}_{3i}^I)}$ and the estimated standard error:

$$\text{se}(\bar{\beta}_3^I) = \sqrt{\frac{1}{\sum_{i=1}^k 1/\text{var}(\widehat{\beta}_{3i}^I)}}.$$

The test statistic $T_{INT} = \left(\frac{\bar{\beta}_3^I}{\text{se}(\bar{\beta}_3^I)} \right)^2$ can be compared it to a one degree of freedom chi-square distribution to test if β_3^I is equal to zero.

Screening by Main Effects

One strategy for detecting SNP $\times$ E interactions when many SNPs are being tested is to assess interaction in the subset of SNPs with significant main effects [Kooperberg 2008]. Here, we perform the meta-analysis of the main effects and carry out the meta-analysis of the interaction term among those SNPs with a main effects p-value less than a pre-specified threshold α_M . This limits the number of interaction tests performed, and therefore permits the use of a less stringent α -level correction. This approach has the theoretical advantage of increasing power under the assumption that the SNPs with strong interaction effects will also have marginal effects which pass the screening step. Two values of α_M will be considered: 0.01 and 0.05.

To control the type I error rate, a critical assumption is that the tests at the first stage are independent of the tests at the second stage. A proof of this appears in Kooperberg [2008]. The type I error rate of α of all N SNPs is preserved by allowing $N_M = \alpha_M \times N$ SNPs to pass to the second stage. The number of null SNPs reaching statistical significance at the second stage, with significance threshold of α/N_M , is then $N_M \times \alpha/N_M = N \times \alpha$.

Meta-analysis of 2 Degree of Freedom Test

Kraft et al. [2007] proposed a 2 degree of freedom (2 *df*) test of main and interaction effects in the context of genome-wide association studies (GWAS) which detects SNPs with main effects and SNPs with heterogeneity in genetic effects across different levels of the environmental variable.

Within each study, the 2 *df* test statistic can be constructed: $T_i = (\hat{\beta}_i^I)^T (\sum_i^I)^{-1} \hat{\beta}_i^I$, where $\hat{\beta}_i^I = (\hat{\beta}_{2i}^I, \hat{\beta}_{3i}^I)$ and $\sum_i^I = \text{cov}(\hat{\beta}_i^I)$. This statistic is compared to a 2 *df* chi-square distribution to test if both $\beta_{2i}^I = 0$ and $\beta_{3i}^I = 0$.

The p-value of this test, p_i , can then be meta-analyzed using Fisher's method [Hedges and Olkin 1985], which provides a summarized chi-square statistic with $2k$ degrees of freedom:

$$T_{MA2df} = -2 \sum_{i=1}^k \log(p_i)$$

Although this method meta-analyzes a joint test of the SNP and SNP $\times$ E beta coefficients, summary estimates of the SNP and SNP $\times$ E beta coefficients are not produced. Joint summary estimates are desirable in order to interpret the association between the SNP and the outcome in the possible presence of interaction between the SNP and E. Moreover, Fisher's method does not take the direction of the beta coefficients into consideration.

Simulated Data

We simulated data from 5 studies with 1,000 participants each. The simulated data included a continuous environmental variable, *E*, a SNP with minor allele frequency of 0.30 and a quantitative trait, *Y*, whose mean value is a function of *E* and the SNP. The effect of the SNP was additive so that the mean value of *Y* is higher for those with more copies of the minor allele. The continuous environmental variable, *E*, explained 10% of the variation in *Y*, while the main effect of the SNP explained between 0% and 1% of the variation in *Y* and the interaction between *E* and the SNP explained between 0% and 1% of the variation in *Y*. In addition to the SNP associated with *Y*, 999 non-associated SNPs with minor allele

frequencies uniformly between 0.05 and 0.5 were generated. A total of 15 scenarios were considered, with 1,000 simulations each.

In the context of multiple independent tests, a standard α -level correction can be used to determine statistical significance. If N denotes the number of SNPs tested, a Bonferroni correction of α/N can be used to define the significance threshold for an overall type I error rate of α . This controls the family-wise error rate, defined as the probability of having one or more false positive associations in each simulation, at nominal level α . For the screening method, a threshold α_M must be defined. For these simulations, we set α_M to 0.01 and 0.05, which translates to carrying forward 10 or 50 null SNPs to the second stage. If N_M denotes the number of SNPs passing the first stage, a Bonferroni correction of α/N_M can be used to define statistical significance at the second stage.

Type I Error and Power

The empirical type I error rate of each method was assessed by first observing the proportion of significant null SNPs within each simulation, using a corrected significance threshold of $0.01/999=1.001\times 10^{-5}$ for each set of 999 null SNPs. These proportions are averaged across all simulations. The theoretical type I error rate for each simulation is 0.01. The power of each method was determined by observing the proportion of simulations in which the associated SNP was statistically significant.

Results

Type I Error

The comparison of the empirical type I error rates in 15,000 simulations is displayed in Figure 1a. The theoretical error rate is 0.01, as each of the simulations had 999 null SNPs with a Bonferroni corrected significance level of $0.01/999=1.001\times 10^{-5}$. The empirical type I error rate of all 14,985,000 null SNPs and the breakdown of empirical type I error rates by minor allele frequency category are displayed in Figures 1b and 1c, respectively. These rates are reported on the SNP level, where the theoretical type I error rate is 1.001×10^{-5} . All five methods had an empirical type I error rate with 95% confidence intervals overlapping the theoretical type I error rate indicated by a horizontal line, except the joint meta-analysis in the 0.20–0.25 minor allele frequency category, which had a slightly inflated empirical type I error rate.

Power

The five methods described here have different null hypotheses, resulting in differences in power when the effects of the SNP and the SNP $\times$ E terms are varied. The meta-analysis of the main effect tests the null hypothesis that $\beta_2^M=0$, and the meta-analysis of the interaction effect tests the null hypothesis that $\beta_3^I=0$. The screening approach tests the null hypothesis that $\beta_3^I=0$ for SNPs whose main effect meta-analysis p-value is less than α_M . Finally, both the meta-analysis of the 2 *df* test and the joint meta-analysis (JMA) test the null hypothesis that $\beta_2^I=0$ and $\beta_3^I=0$, although the meta-analytic methods differ.

A comparison of the power of the five methods is presented in Figure 2. When there is no interaction effect, the main effects meta-analysis, the meta-analysis of the 2 *df* test and the joint meta-analysis can be compared in terms of power, as these methods test the main effect of the SNP. Figure 2a describes the scenario where the percent variance in Y explained by the SNP is increased from 0% to 1% and the percent of variances of Y explained by SNP $\times$ E is 0%. The meta-analysis of the main effect has the highest power, with the joint meta-analysis having slightly less power.

When there is an interaction effect, the interaction meta-analysis and the screening approach can be added to the power comparison. Figures 2b and 2c show scenarios where the percent of variance of Y explained by $\text{SNP} \times E$ is 0.5% and 1%, respectively. In these situations, the joint meta-analysis has the highest power, except in the situation where there is no main effect (i.e. the percent variance explained by the SNP is zero), in which case the meta-analysis of the interaction alone has the highest power. When α_M was set to 0.05, a more generous screening threshold, power for the screening method was decreased (data not shown) as more SNPs passed the screening and the significance threshold for the interaction analysis was lowered.

Implementation in a large genetic consortium

The missense P12A variant within the *PPARG* gene has been found to be associated with type 2 diabetes [Altshuler 2000], a phenotype related to insulin resistance and impaired beta-cell function. *PPARG* encodes the peroxisome proliferator activated receptor $\gamma 2$, a transcription factor involved in adipocyte differentiation and the target of thiazolidinedione medications. It is believed that the risk allele at *PPARG* P12A increases risk of type 2 diabetes by reducing insulin sensitivity. The covariate Body Mass Index (BMI) is strongly associated with type 2 diabetes; individuals with higher BMI are at higher risk of the disease, and BMI may modify the risk conferred by the *PPARG* P12A variant [Florez 2007, Ludovico 2007, Tonjes 2006]. We assessed the association of 60 tagSNPs within 1,000 kb of *PPARG* with the diabetes-related quantitative trait of fasting insulin levels (as a surrogate of insulin resistance) in 5 cohorts, with sample sizes ranging between 561 and 8,367 non-diabetic participants, resulting in a sample size of 19,466 individuals. The cohorts, described in Table 1, are: the Framingham Heart Study [Meigs 1998] (FHS), the Family Heart Study [Higgins 1996, Coon 2004] (FamHS), the Cardiovascular Heart Study [Fried 1991, Cushman 1995, Psaty 2009] (CHS), the Atherosclerosis Risk in Communities Study [ARIC 1989] (ARIC) and the Dynamics of Health, Aging and Body Composition Study [Simonsick 2001] (Health ABC). The outcome trait, fasting insulin, was naturally log-transformed in order to reduce the skewness of its distribution.

The $-\log_{10}(\text{p-values})$ of the meta-analysis of each of the 60 tagSNPs within 1000 kb of the *PPARG* gene are presented in Figure 3. The beta estimates and p-values for SNPs reaching statistical significance are listed in Table 2 for all methods except the screening approach. The significance threshold was $\alpha/N = 0.01/60 = 1.67 \times 10^{-4}$. We did not detect significant heterogeneity in the regression slopes among the five samples ($p > 0.01$). Significant associations were seen with both the meta-analysis of the 2 *df* test and the joint meta-analysis. The most significant SNP, rs1801282 (*PPARG* P12A), had a p-value of 1.15×10^{-6} with the meta-analysis of the 2 *df* test and 8.29×10^{-9} with the joint meta-analysis. Neither the main effects meta-analysis nor the interaction meta-analysis produced tests with p-values less than the corrected significance threshold of $0.01/60 = 0.000167$. For the screening approach, even though the main effects meta-analysis would have allowed the SNP to pass to the second stage with a p-value below $\alpha_M = 0.01$, the p-value of the interaction meta-analysis would not have been below the corrected p-value threshold of α/N_M .

The effect of the SNP rs1801282 (*PPARG* P12A) on $\log(\text{Insulin})$ for BMI values of 25, 30 and 35 are displayed in Table 3. For a BMI of 25, the effect of the risk allele of rs1801282 on $\log(\text{Insulin})$ is 0.041 (se = 0.0073), while for a BMI of 35, the effect is 0.057 (se = 0.016), demonstrating the synergistic effect of BMI on the association between *PPARG* P12A and $\log(\text{Insulin})$.

Discussion

We have shown that the joint meta-analysis (JMA) has higher power than the other methods when both main and interaction effects are present. Most of the difference in power between the methods can be attributed to the different hypotheses and to the different degrees of freedom in the tests. The main effects meta-analysis and the interaction meta-analysis have one degree of freedom, the meta-analysis of the 2 *df* test has 2*k* degrees of freedom and the JMA has 2 degrees of freedom. As demonstrated in Figure 2a, when comparing the ability of the three methods to detect the main effect in the absence of interaction, the main effects meta-analysis has the highest power, followed closely by the JMA. The meta-analysis of the 2 *df* test has 10 degrees of freedom, resulting in lower power than the other two methods.

The joint meta-analysis strategy simultaneously tests and estimates the SNP and SNP×E beta coefficients of an interaction regression model. This method accounts for the covariance between the SNP beta estimate and the SNP×E beta estimate. A joint test of significance is available, testing the null hypothesis that both the SNP beta and SNP×E beta are equal to zero. A confidence region of the beta estimates can be constructed using the covariance matrix of the meta-analyzed beta estimates. Finally, we provide a test of heterogeneity of the beta estimates. The meta-analyzed beta coefficients and the joint test fully characterize the association between the SNP and the outcome, allowing for a possible interaction between the SNP and the environmental variable. We have confirmed that *PPARG* P12A interacts with BMI in increasing insulin resistance, a phenomenon which may be extended to other putative insulin resistance loci.

The joint meta-analysis method has been implemented in METAL available from <http://www.sph.umich.edu/csg/abecasis/Metal>. Individual studies can obtain the required beta and covariance estimates for interaction regression models using QUICKTEST version 0.95 (<http://toby.freeshell.org/software/quicktest/>) and ProbABEL version 0.1–3 (<http://mga.bionet.nsc.ru/yurii/ABEL/>) [Aulchenko 2007].

We hope that the use of this method in appropriately sized consortia with relevant environmental variables will lead to both the identification of novel associations with complex quantitative traits and a more refined characterization of existing signals. When an environmental variable is known or suspected to contribute to the expression of complex quantitative traits and phenotypes, a greater proportion of the heritability of complex traits may be explained by accounting for the joint effects of the SNP and the SNP-environment interaction.

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FHS

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Figure 1.

(A) Empirical type I error rate in 15,000 simulations of 999 null SNPs, empirical type I error rates for (B) 14,985,000 null SNPs and (C) 14,985,000 null SNPs broken into minor allele frequency categories. A horizontal line is drawn at the theoretical type I error rate in each plot.

Figure 2.

Power of the five methods of meta-analysis compared in the simulation study. Both the percent of the variance in the outcome explained by the SNP and the SNP $\times$ E terms vary between 0% and 1%.

PPARG region: rs1801282

Figure 3.

Single SNP associations of SNPs in the *PPARG* gene with log(fasting insulin) in a meta-analysis of 5 studies of 19,466 non-diabetic individuals, after taking into account a putative interaction with BMI. The *PPARG* P12A SNP is indicated by a double-diamond and annotated with the p-value from the joint meta-analysis. The shading represents SNPs with R^2 greater than 0.2 with P12A.

Cohorts participating in the meta-analysis of the association between log(fasting insulin) and SNPs in the PParG gene with possible interaction with BMI.

Table 1

Cohort	Sample Size	Age Mean (SD)	% Female	Body Mass Index Mean (SD)	Fasting Insulin (pmol/l) Mean (SD)
CHS	2,854	72.3 (5.4)	57%	26.0 (4.3)	93.9 (49.1)
ARIC	8,367	54.1 (5.7)	54%	26.7 (4.6)	72.1 (53.9)
FamHS	561	53.4 (11.9)	47%	27.7 (5.1)	69.1 (43.8)
FHS	6,023	46.2 (11.6)	54%	27.0 (5.0)	29.9 (16.5)
Health ABC	1,661	73.8 (2.8)	47%	26.6 (4.1)	57.0 (40.6)
Total	19,466				

Table 2

Results of meta-analysis of 3 SNPs from the *PPARG* gene attaining statistical significance of $p < 0.01/60=0.000167$ with the meta-analytic methods assessing the association of the SNP and/or the SNP×BMI regression coefficients from the main effect and interaction regression models.

SNP	Main Effects Meta-Analysis		Interaction Meta-Analysis		Meta-Analysis of the 2 df Test			Joint Meta-Analysis			Test of Homogeneity	
	$\hat{\beta}_2^M$	p	$\hat{\beta}_3^I$	p	T_{2df} (8 df)	p		$\hat{\beta}_2^I$	$\hat{\beta}_3^I$	T_{JMA} (2 df)	p	p (8 df)
rs1801282	0.0293	0.001	0.0013	0.44	46.52	1.15×10^{-6}		0.0009	0.0016	37.22	8.3×10^{-9}	0.317
rs2067819	-0.0205	0.005	0.0002	0.86	37.34	4.94×10^{-5}		-0.0237	-0.0002	24.37	5.1×10^{-6}	0.113
rs2120825	0.0216	0.050	0.0005	0.80	27.33	2.31×10^{-3}		0.0232	0.0006	22.2	1.5×10^{-5}	0.743

Table 3

The effect of rs1801282 (*PPARG* P12A) on log(fasting insulin) for three values of BMI from the joint meta-analysis.

BMI	Effect	SE(Effect)
25	0.041	0.0073
30	0.049	0.0094
35	0.057	0.0160