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An Extension of the Regression of Offspring on Mid-Parent to Test for Association and Estimate Locus-Specific Heritability: The Revised ROMP Method

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Summary

The Regression of Offspring on Mid-Parent (ROMP) method is a test of association between a quantitative trait and a candidate locus. ROMP estimates the trait heritability and the heritability attributable to a locus and requires genotyping the offspring only. In this study, the theory underlying ROMP was revised ($ROMP_{rev}$) and extended. Computer simulations were used to determine the type I error and power of the test of association, and the accuracy of the locus-specific heritability estimate. The $ROMP_{rev}$ test had good power at the 5% significance level with properly controlled type I error. Locus-specific heritability estimates were, on average, close to simulated values. For non-zero locus-specific heritability, the proposed standard error was downwardly biased, yielding reduced coverage of 95% confidence intervals. A bootstrap approach with proper coverage is suggested as a second step for loci of interest.

$ROMP_{rev}$ was applied to a study of cardiovascular-related traits to illustrate its use. An association between polymorphisms within the fibrinogen gene cluster and plasma fibrinogen was detected ($p < 0.005$) that accounted for 29% of the estimated fibrinogen heritability. The $ROMP_{rev}$ method provides a computationally fast and simple way of testing for association and obtaining accurate estimates of locus-specific heritability while minimizing the genotyping required.

Keywords: association tests, quantitative trait, parent-offspring trios, candidate gene, cardiovascular disease

Introduction

Testing for associations between polymorphisms and quantitative traits provides a tool that can help to identify the genetic effects underlying multifactorial disease. An association between a genetic marker and a quantitative trait indicates that the genetic marker may be a functional polymorphism responsible for part of the variation in the trait, or in linkage disequilibrium with a functional polymorphism situated nearby. In addition to testing for association, the ability to estimate how much of the variation in a quantitative trait is attributable to variation at a given marker locus, i.e. the heritability attributable to the locus (or the locus-specific heritability), can help to assess the importance of a polymorphism found through association testing, and may help to prioritize subsequent studies. Locus-specific heritability can be used in a manner similar to that of attributable risk in assessing what proportion of the disease in the population is attributable to a risk factor. This can be especially helpful in studying the etiology of multifactorial disease in which several loci and environmental factors are likely to play a role.

The approaches that can be used to test for association between a quantitative trait and a marker locus can be classified into two broad categories: population-based and intrafamilial tests. Population-based tests of association use samples of unrelated individuals drawn from the population, such as case-control designs for qualitative traits. For quantitative traits, statistical tests – like one-way analysis of variance (ANOVA) – can be used to compare the means of the trait among individuals with different alleles or genotypes at

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the locus. The variation in the trait attributable to the locus can be estimated with this approach using the mean square associated with the marker locus effect. However, this estimate ignores the presence of genetic effects other than the locus included in the ANOVA since familial relationships are not included. Locus-specific heritability estimates from unrelated individuals are dependent on accurate specification of the genetic model, particularly with respect to the specification of accurate allele frequencies.

At the other end of the spectrum are the intra-familial tests of association. The transmission/disequilibrium (TDT) test (Spielman et al. 1993) was introduced for qualitative traits and uses information about the transmission of alleles from parent to offspring to test for combined association and linkage. Several TDT tests for quantitative traits have been developed (Allison, 1997; Rabinowitz, 1997; Xiong et al. 1998). Intra-familial tests of association and linkage have also been developed in likelihood-based, variance components contexts (Abecasis et al. 2000; Fulker et al. 1999). Likelihood-based tests of association that do not use allele sharing have also been proposed, including the measured genotype approach (Boerwinkle et al. 1986) and likelihood-based pedigree tests of association (George & Elston, 1987). Likelihood-based tests take into account the most information; their ability to provide an estimate and a test of both the heritability of the trait and the heritability attributable to the single-locus effect makes these methods quite attractive.

Pugh et al. (2001) proposed a method that shares the advantages of both population-based and intra-familial likelihood-based methods. The Regression of Offspring on Mid-Parent (ROMP) method is an extension of the linear regression of offspring on mid-parent value traditionally used to estimate the heritability of a trait. With the inclusion of a candidate locus effect as a covariate in the regression, ROMP allows the investigation of single-locus and multi-locus effects. ROMP is similar to the TDT in the sense that data on offspring and their parents are utilized: the TDT uses the transmission of genotypic data from parents to offspring, while ROMP uses the correlation between the phenotypes of offspring and parents. Hence, while the TDT requires genotypic data on the parents and genotypic and phenotypic data on the offspring, ROMP requires phenotypic data on the parents and the offspring and genotypic data only on the offspring. Like the computationally intensive likelihood-based methods, ROMP can be used to estimate and test the significance of the heritability of the trait, test for a single-locus effect (i.e. test for a locus-trait association) and estimate the heritability attributable to the candidate locus. Like the population-based methods, ROMP is fast and computationally simple but is not generally robust to population stratification.

Association tests are often used in the fine mapping of large candidate regions indicated by linkage analysis of a quantitative trait. With the increasing availability of large numbers of Single-Nucleotide Polymorphisms (SNPs) (Wang et al. 1998) and the completion of the HapMap project (The International HapMap Consortium, 2005), a direct search for associations between a trait and SNP markers is now quite practical. The genotyping cost required to identify an association for a quantitative trait is an important consideration in the study design. ROMP minimizes the amount of genotyping required while still providing an estimate of the heritability attributable to the single-locus effect.

The original version of ROMP (Pugh et al. 2001) used the difference in slopes between regressions with and without a marker locus as the locus-specific heritability estimate; however, this estimate was found to be downwardly biased. In this paper, the expected value of the original ROMP locus-specific heritability estimator is derived, allowing the bias to be quantified. A revised estimator for the locus-specific heritability that corrects for this bias is introduced along with a large-sample variance and a bootstrap estimate of the variance. Simulation experiments are used to investigate the statistical properties of this revised ROMP method (denoted as ROMP_{rev}).

To illustrate the method, ROMP_{rev} was applied to a study of cardiovascular-related traits in a Korean population. Associations between candidate polymorphisms and plasma levels of hemostatic factors were investigated. Plasma levels of hemostatic factors have been found to be risk factors for atherosclerotic cardiovascular disease in several epidemiological studies (Folsom, 2001). Hemostatic factors, including fibrinogen, coagulation factor VII and plasminogen activator inhibitor – 1 (PAI –1), have been shown to be heritable with heritability estimates ranging between 30 and 70% (Cesari et al. 1999; de Lange et al. 2001; Freeman et al. 2002; Souto et al. 2000). Polymorphisms located in the fibrinogen, factor VII and PAI –1 genes have been reported to be associated with plasma levels of these factors (Grant & Humphries, 1999; Lane & Grant, 2000). Quantifying the contribution of these polymorphisms to the heritability of the traits in different populations may help to understand the mode of inheritance of the traits and guide in the search for other risk factors.

Materials and Methods

The revised Regression of Offspring on Mid-Parent (ROMP_{rev}) method

Suppose that a quantitative trait y is a linear function of three independent effects: a locus effect l, a polygenic effect P and a

residual effect e, such that individual i has trait value $y_i = \mu + l_i + P_i + e_i$, where μ is the overall mean. The genotypic value, l_i, is the effect on the trait associated with the individual's genotype at locus L. The variance of the trait is $\sigma_y^2 = \sigma_l^2 + \sigma_P^2 + \sigma_e^2$. The additive genetic variance, $\sigma_a^2 = \sigma_{a_l}^2 + \sigma_{a_P}^2$, is used in the estimation of the narrow sense heritability of the trait. The heritability in the narrow sense, simply called heritability hereafter, is the ratio of the additive genetic variance to the phenotypic variance: $h^2 = \sigma_a^2/\sigma_y^2$. In a population-based sample of parent-offspring trios, a traditional estimate of heritability can be obtained from the linear regression of the offspring's phenotype, y_o, on the average phenotype of his parents, the mid-parent value: $y_{mp} = (y_f + y_m)/2$, where y_f and y_m represent the father's and mother's phenotypes, respectively (Falconer & Mackay, 1996; Fisher, 1918; Kempthorne & Tandon, 1953). Assuming, without loss of generality, that the variables are centered around the sample means, this regression can be written as

$$y_o = \beta_{o,mp}\, y_{mp} + \varepsilon, \tag{1}$$

where ε is a normally distributed residual effect. Assuming that the relationship between y_o and y_{mp} is truly linear, the regression coefficient $\beta_{o,mp}$ is equal to the offspring-mid-parent covariance divided by the mid-parent variance. Assuming random mating, no selection, no gene-environment interaction and no parental environmental effects, it can be shown that the offspring-mid-parent covariance is one-half the additive genetic variance, i.e., $\sigma_{y_o, y_{mp}} = \sigma_a^2/2$. The variance of the mid-parent value is one-half the phenotypic variance, i.e., $\sigma_{y_{mp}}^2 = \sigma_y^2/2$, so that $\beta_{o,mp} = \sigma_a^2/\sigma_y^2$ (Lynch & Walsh, 1998). Hence, the estimate of the slope of the regression of offspring phenotype on mid-parent phenotype is an estimate of the heritability (h^2) of the trait.

As noted in Pugh et al. (2001), the effect of a locus can be added to the above regression model by including the genotype of the offspring at the locus, g_o, as a covariate in the model:

$$y_o = \beta_r\, y_{mp} + \gamma_l g_o + \nu, \tag{2}$$

where ν is a normally distributed residual effect. The genotype of the offspring is entered in the regression model assuming an additive allelic effect, i.e., g_o equals 0, 1, or 2 for the number of copies of the 'variant' allele. Using equation (2), a test of the locus effect on the trait can be obtained by testing whether its coefficient, γ_l, equals zero. If the locus is responsible, at least in part, for the variation of the trait, the coefficient of y_{mp} will be reduced in model (2) compared to model (1).

With the assumptions noted above, it can be shown that the coefficient of the mid-parent value in model (2) is $\beta_r = \sigma_{a_P}^2/(\sigma_y^2(1 - h_l^2/2))$, where $h_l^2 = \sigma_{a_l}^2/\sigma_y^2$ is the locus-specific heritability (see Supplementary Appendix I for details). Because $\beta_{o,mp} = \sigma_a^2/\sigma_y^2$, an estimator of h_l^2 can be obtained from $\beta_{o,mp}$ and β_r by noting that

$$h_l^2 = \frac{\sigma_{a_l}^2}{\sigma_y^2} = \frac{\sigma_a^2 - \sigma_{a_P}^2}{\sigma_y^2}, \tag{3}$$

and that the difference between $\beta_{o,mp}$ and β_r is

$$\beta_{o,mp} - \beta_r = \frac{\sigma_a^2}{\sigma_y^2} - \frac{\sigma_{a_P}^2}{\sigma_y^2\left(1 - h_l^2/2\right)}. \tag{4}$$

When there is only one locus responsible for some of the variation in the trait, $\sigma_{a_P}^2 = 0$, $h_l^2 = h^2$, and the difference in slopes, $\beta_{o,mp} - \beta_r$, is equal to the heritability attributable to the locus. However, when more than one locus determines the trait, the difference in slopes is a biased estimator of h_l^2. Using equation (3) and (4), solving for $h_l^2$and replacing the regression coefficients by their usual least-squares estimators, the estimator of h_l^2 is

$$\hat{h}_l^2 = \frac{\hat{\beta}_{o,mp} - \hat{\beta}_r}{1 - \hat{\beta}_r/2}. \tag{5}$$

Conditioning on y_{mp} and g_o and assuming that the errors from the regression model (2) are normally distributed with mean 0 and variance σ_ν^2, the locus-specific heritability estimator is a function of two normally distributed random variables: $\hat{\beta}_{o,mp}$, and $\hat{\beta}_r$. The variance of $\hat{h}_l^2$ can be approximated by using the delta method (see Supplementary Appendix II for details):

$$\mathrm{Var}\left(\hat{h}_l^2\right) \approx \frac{(1 - \beta_r/2)\,(\beta_{o,mp} - \beta_r/2 - 1)\,\mathrm{Var}(\hat{\beta}_{o,mp}) + (1 - \beta_{o,mp}/2)^2\,\mathrm{Var}(\hat{\beta}_r)}{(1 - \beta_r/2)^4}, \tag{6}$$

where

$$\mathrm{Var}(\hat{\beta}_{o.mp}) = \frac{\sigma_\nu^2}{\sum y_{mp}},$$

and

$$\mathrm{Var}(\hat{\beta}_r) = \frac{\sigma_\nu^2 \sum g_o^2}{\sum y_{mp}^2 \sum g_o^2 - (\sum y_{mp} g_o)^2}.$$

An estimate of $\mathrm{Var}(\hat{h}_l^2)$ can be obtained by replacing, in equation (6), the coefficients $\beta_{o,mp}$ and β_r by their least-squares estimates, and σ_ν^2 by the residual sum of squares from regression (2) divided by its degrees of freedom, $n - 3$. This estimated variance can then be used to obtain a test of significance for the locus-specific heritability estimate, based on a t distribution with $n - 3$ degrees of freedom because $\hat{h}_l^2/\hat{\sigma}_{\hat{h}_l^2}$ approximately follows a t_{n-3} under the null hypothesis that $h_l^2 = 0$. A 95% confidence interval can also be obtained by taking $\hat{h}_l^2 \pm t_{0.975,n-3}\hat{\sigma}_{\hat{h}_l^2}$.

Thus, the ROMP$_{\mathrm{rev}}$ method provides the following: 1) estimates of the heritability of the trait and of its standard error ($\hat{h}^2 \pm \hat{\sigma}_{\hat{h}^2}$), and a test of the null hypothesis that $h^2 = 0$ by using the traditional regression of offspring on mid-parent (Falconer & Mackay, 1996); 2) estimates of the heritability attributable to a locus and of its standard error ($\hat{h}_l^2 \pm \hat{\sigma}_{\hat{h}_l^2}$) by adding a locus effect in the regression model as described above; and 3) a test of association between the trait and a locus by testing either the

null hypothesis that $\gamma_l = 0$, or that $h_l^2 = 0$. Clogg et al. (1992) showed that testing for $\gamma_l = 0$ was equivalent to testing for $\beta_{o,mp} - \beta_r = 0$, when the latter was done conditional on γ_{mp} and g_o. The test for $h_l^2 = 0$ based on $\hat{h}_l^2$ from equation (5) is derived in a similar manner as the test described in Clogg et al. (1992) and will thus theoretically have a similar behavior. It should be slightly more powerful in the specific case of testing for significant locus-specific heritability since it corrects for the inherent bias of $\beta_{o,mp} - \beta_r$. We verified empirically, in a subset of our simulations, that testing for $\gamma_l = 0$ and testing for $h_l^2 = 0$ based on $\hat{h}_l^2/\hat{\sigma}_{\hat{h}_l^2}$ were equivalent (data not shown). Since our goal was to test that $h_l^2 = 0$, we chose to use $\hat{h}_l^2/\hat{\sigma}_{\hat{h}_l^2}$ to test for association in ROMP_{rev}.

The trait can easily be adjusted for relevant covariates by performing a regression with all available data (parents as well as offspring) and then using the residuals of this regression as the trait in the ROMP_{rev} analysis. Regression diagnostics can also be used to examine trait phenotypes for outliers. Stepwise and multiple regression can be used to identify significant single-locus effects from the substantial number of loci and/or functional SNPs that may lie within candidate regions identified with linkage analysis.

Simulations

Simulations were used to investigate the type I error rate and the power of the ROMP_{rev} method to detect a single-locus effect and to evaluate the accuracy of the estimates of the locus-specific heritability. The Genometric Analysis Simulation Program (G.A.S.P. version 3.3, (Wilson et al. 1996)) was used to simulate a quantitative trait determined by a two-allele single-locus effect, a polygenic effect and a residual effect with additive effects. Different models with varying locus-specific heritability were used to generate the data. The total trait heritability and the residual variability were each fixed to 50%, while the different values for the locus-specific heritability were 0, 1, 5, 10, 20, 30, and 50%. The polygenic heritability was set as the remaining heritability, i.e., $h_P^2 = h^2 - h_l^2$. Two sets of simulations were performed with different minor allele frequencies (MAF) of the allele at the locus responsible for variation in the trait: a common allele (MAF = 0.25) and a rare allele (MAF = 0.05).

Samples of 150 independent parent-offspring trios were simulated with complete phenotypic data and genotypic data at the locus. Two-thousand replicates were generated for each model, providing a 95% confidence interval of maximum length 0.02 for estimating the probability of type I error. Simulations were also performed for a sample size of 50 nuclear families with three offspring. These 50 nuclear families were analyzed in two ways: 1) by duplicating the parental data for each offspring, yielding a sample size of 150 non-independent trios; and 2) by choosing one offspring at random from each family, yielding a sample size of 50 independent trios. All analyses were performed assuming independent trios. Results from the 150 non-independent trios were compared to those from the 150 independent trios to evaluate the effect of the non-independence of trios on the type I error of the ROMP_{rev} test of association. Results from the 50 independent trios were compared to those from the 150 independent trios to investigate the effect of sample size.

Three approaches providing a standard error and/or a test of significance for the locus-specific heritability estimate were compared: the parametric (PAR) ROMP_{rev} method as described above, a nonparametric bootstrap (BOOT) approach and a permutation test (PERM) approach. The parametric standard errors were compared to their Monte Carlo estimates from the 2000 replicates and to bootstrap standard errors. Bootstrap samples consisted of 1000 samples with replacement of trios $(\gamma_{o_i}, \gamma_{mp_i}, g_{o_i})$. Average lengths and estimated coverage probabilities of confidence intervals were also compared between the parametric and bootstrap approaches. The bootstrap 95% confidence interval was used, i.e., the 2.5 and 97.5 percentiles of the bootstrap distribution were used as confidence limits (Efron & Tibshirani, 1993).

Estimated probability of type I error and power at the 0.05 level were compared for the parametric, the bootstrap and the permutation test approach. The bootstrap test was based on the bootstrap confidence interval, i.e., the null hypothesis was rejected whenever the bootstrap confidence interval did not include 0. For the permutation test of the null hypothesis that $h_l^2 = 0$, the phenotypes of the offspring and parents, $(\gamma_{o_i}, \gamma_{mp_i})$, were kept together, while genotypes of the offspring were permuted. The locus-specific heritability estimator was used as the test statistic in one thousand permuted samples to obtain an empirical p-value (Good, 2000).

The parametric, bootstrap and permutation test approaches for ROMP_{rev} were implemented with the R software (Ihaka & Gentleman, 1996).

Application to a study of cardiovascular disease

ROMP_{rev} was used to determine the contribution of known candidate polymorphisms to the heritability of cardiovascular-related traits in 87 Korean families (508 individuals). These families were previously described (Jee et al. 2002a,b) and were ascertained through probands undergoing elective coronary arteriography as part of the Yonsei Coronary Artery Disease Study. The families were mostly two and three-generation families with a median number of 6 phenotyped individuals per family. Study protocols were approved by the Ethics Committee of Severance Hospital, Yonsei University and informed consent was obtained from each of the study subjects.

Blood samples were obtained for the measurement of plasma levels of hemostatic factors and for the genotyping of the DNA polymorphisms. The polymorphisms were candidates for the different hemostatic factors: the Arg353Gln polymorphism located in the gene encoding coagulation factor VII (Green et al. 1991); three linked polymorphisms (HaeIII, AluI and MnlI) located within the fibrinogen gene cluster (Baumann & Henschen, 1994); and the 4G/5G insertion/deletion located in the promoter of the gene encoding plasminogen activator inhibitor – 1 (PAI –1) (Dawson et al. 1993). Minor allele frequencies of the polymorphisms ranged from 0.09 to 0.48.

ROMP_{rev} was applied to this sample of families, by choosing one trio per family at random among trios with complete data for ROMP_{rev}. This yielded 56 to 58 independent trios depend-

ing on the trait. The results from $ROMP_{rev}$ were compared to results obtained from the family-based test of association, as implemented in the FBAT software (Rabinowitz & Laird, 2000), to the orthogonal quantitative trait transmission-disequilibrium test implemented in the QTDT software (Abecasis et al. 2000) and to the George and Elston (1987) test as implemented in ASSOC of the S.A.G.E. package (S.A.G.E., 2002). The empirical variance was used in the FBAT analysis (Lake et al. 2000). Identity-by-descent (i.b.d.) sharing was estimated using SIMWALK2, in order to use the orthogonal model of Abecasis et al. (2000) within a variance components framework. For QTDT, FBAT and ASSOC, analyses were performed on the selected, independent trios (in order to compare the different methods using the same sample) as well as on the full family structure. Adjustment for age, sex, smoking status and drinking status was made for all analyses. Smoking status was entered in the model as two dichotomous variables: one for current smoking and one for past smoking. Drinking status was analyzed in the same way.

Results

Simulations

Figure 1 is a summary of the accuracy of the estimation of the locus-specific heritability by $ROMP_{rev}$ with 150 independent trios. Figure 1A shows the mean locus-specific heritability and its standard deviation calculated over the 2000 replicates. The standard deviation of the estimates is the Monte Carlo estimate of the standard error and was the basis to evaluate the parametric and bootstrap standard errors. Figure 1A also presents the standard errors, averaged over the replicates, obtained from the parametric and bootstrap approaches. Figure 1B presents the estimated coverage probability of the 95% confidence interval, i.e., the probability that the interval covers the true locus-specific heritability, for the parametric and bootstrap approaches. Average estimates of the locus-specific heritability were very close to the simulated h_l^2 for all generating models (Figure 1A, bar heights). For the common allele case (MAF $= 0.25$), the estimated relative bias, i.e., the average error divided by the simulated h_l^2, ranged from -0.6% to 0.5% (for $h_l^2 = 0.01$ and 0.5, respectively). For the rare allele case (MAF $= 0.05$), the relative bias ranged from -0.2% to 1.9% (for $h_l^2 = 0.5$ and 0.01, respectively). The bias of the estimator was negligible relative to its standard error. The estimated bias was on average 125 times smaller than the standard deviation of the estimates.

For simulated $h_l^2 = 0$, parametric standard errors were close to their Monte Carlo estimate, while the bootstrap standard errors were larger (Figure 1A, error bars). The estimated coverage probability of the parametric 95% confidence interval was close to 95% (95.3% for MAF $= 0.25$, 96.5% for MAF $= 0.05$; Figure 1B). Bootstrap intervals had an average length twice as wide as the length of the parametric intervals, yielding an estimated coverage probability over 99.5% (Figure 1B). When $h_l^2 > 0$, parametric standard errors were smaller than the Monte Carlo estimates (on average 1.6 times for MAF $= 0.25$ and 1.8 times for MAF $= 0.05$; Figure 1A), yielding a narrow 95% confidence interval length with a coverage probability smaller than 95% (Figure 1B). The estimated coverage probability was on average $73 \pm 2\%$ for MAF $= 0.25$ and $68 \pm 8\%$ for MAF $= 0.05$. Coverage worsened as the locus-specific heritability increased towards the total heritability, i.e., as the polygenic component approached 0. The bootstrap estimates were close to the Monte Carlo estimates and bootstrap confidence intervals were twice as large as the parametric intervals but had accurate coverage of $95 \pm 2\%$ for MAF $= 0.25$ and $94 \pm 2\%$ for MAF $= 0.05$ (Figure 1B).

For the common allele case, decreasing the sample size from 150 to 50 independent trios yielded larger standard errors and associated confidence intervals, as expected, but coverage probabilities were similar to those obtained with 150 trios for all models where $h_l^2 > 0$ (data not shown). For the rare allele case, however, lower coverage was observed for both the parametric and bootstrap approaches with average estimated coverage probability of $68 \pm 10\%$ for the parametric approach and $90 \pm 3\%$ for the bootstrap approach. Coverage was over 95% for $h_l^2 = 0$.

For both minor allele frequencies (MAFs), estimated coverage probabilities were reduced when using 150 non-independent trios in the models where $h_l^2 > 0$, while coverage stayed over 95% for $h_l^2 = 0$. Average probabilities for models with $h_l^2 > 0$ were $65 \pm 3\%$ for the parametric approach and $89 \pm 4\%$ for the bootstrap approach with MAF $= 0.25$; and $58 \pm 9\%$ for the parametric approach and $87 \pm 4\%$ for the bootstrap approach with MAF $= 0.05$.

Table 1 presents the estimated probability of type I error ($h_l^2 = 0$ models) and Figure 2 presents the estimated power (models where $h_l^2 > 0$) for the different sample sizes and MAFs. For the parametric approach, the estimates of type I error rates were close to the nominal value of 0.05 when independent trios were analyzed. When 150 non-independent trios were used, the estimated type I error increased to 0.07 for MAF $= 0.25$ and 0.06 for MAF $= 0.05$. The estimated type I error rate of the permutation test ranged from 0.045 to 0.059 when independent trios were analyzed and was equal to 0.09 in the common allele case, and 0.10 in the rare allele case when non-independent trios were analyzed. Estimated type I error rates for the bootstrap were all smaller than 0.03.

The estimated power was highest for the permutation test followed by the parametric approach and the bootstrap (Figure 2). With 150 independent trios, the permutation test could detect a locus-specific heritability of 10%

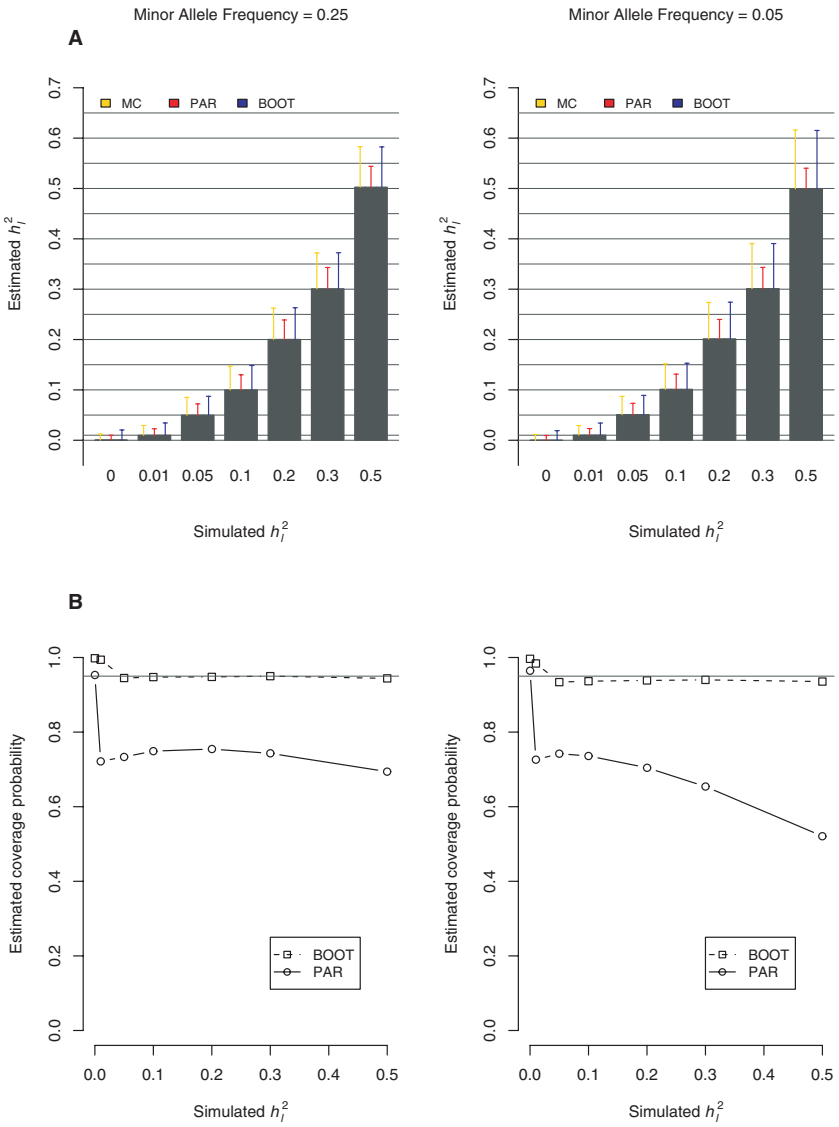

Figure 1 A) $ROMP_{rev}$ estimates of locus-specific heritability, h_l^2 (height of bars) and standard errors averaged over 2000 simulated replicates of 150 independent parent-offspring trios. MC = Monte Carlo estimate of the standard error, i.e., standard deviation of the estimates of h_l^2 among 2000 replicates, PAR = parametric $ROMP_{rev}$ approach, BOOT = bootstrap $ROMP_{rev}$ approach; B) Estimated coverage probability of the 95% confidence interval obtained with the parametric (PAR) and bootstrap (BOOT) approaches. A line is drawn at the 95% coverage probability.

or higher with at least 98% power, while the parametric and bootstrap approaches had 90% and 80% power, respectively. A locus-specific heritability of 5% could be detected with 80, 60, and 40% power with the permutation, parametric and bootstrap approaches, respectively. Power to detect a locus-specific heritability of 10% or larger was reduced to approximately 60, 45, and 25% for the permutation, parametric, and bootstrap approaches, respectively, when 50 independent trios were used. Power obtained with 150 non-independent trios was similar to that obtained with 150 independent trios. Results for the common and rare allele were similar.

Application to a study of cardiovascular disease

Table 2 presents the $ROMP_{rev}$ association results for the adjusted hemostatic factors and their candidate polymorphisms in randomly selected independent parent-offspring

Table 1 Estimated probability of type I error at the 0.05 level for the $ROMP_{rev}$ parametric (PAR), bootstrap (BOOT), and permutation test (PERM) approaches.

	Minor Allele Frequency = 0.25			Minor Allele Frequency = 0.05		
	PAR	BOOT	PERM	PAR	BOOT	PERM
a	0.0470	0.0030	0.0550	0.0355	0.0030	0.0460
b	0.0425	0.0030	0.0445	0.0390	0.0320	0.0585
c	0.0715	0.0140	0.0920	0.0630	0.0170	0.1035

Calculated in 2000 replicates of a) 150 independent parent-offspring trios, b) 50 independent parent-offspring trios, and c) 150 non-independent trios (from 50 nuclear families of size 3).

trios. Fibrinogen was significantly associated with the three linked polymorphisms within the fibrinogen gene cluster ($p < 0.005$). Locus-specific heritability was estimated to be about 4%, accounting for 29% of the total adjusted heritability of fibrinogen. The bootstrap confidence intervals included 0. Analyses of these same data with FBAT, QTDT, and ASSOC also showed a significant association between fibrinogen and the three fibrinogen polymorphisms (Table2; $p < 0.005$). In addition, when the complete family structure was analyzed using FBAT, QTDT, and ASSOC (data not shown), the 4G/5G insertion/deletion was significantly associated with PAI –1 with QTDT ($p = 0.0347$) but not with FBAT or ASSOC ($p > 0.05$), also a significant association was found between factor VII and the Arg353Gln polymorphism with ASSOC ($p = 0.0274$) but not with FBAT or QTDT ($p > 0.1$).

Discussion

In this paper a revised method to test for association with quantitative traits was introduced; the method provides an estimate of the trait heritability and of the heritability attributable to the locus. This $ROMP_{rev}$ method requires genotypic data on offspring only, which substantially reduces the amount of genotyping required to test for associations between a large number of SNPs and quantitative traits. This could be especially useful in cases where it is hard or not feasible to obtain genotypic data on the parents, while phenotypic data is readily available or easy to obtain.

Variance components approaches can be used instead of $ROMP_{rev}$ to obtain a locus-specific heritability estimate in samples of independent parent-offspring trios with parental genotypic data available, in samples of multiple-offspring nuclear families with or without parental data and in samples of extended pedigrees. However, existing variance components methods use identity-by-descent and/or identity-by-state sharing between relatives at the marker locus to test for linkage and/or association. When parental genotypes are not available and only one offspring is available per family, sharing at the marker locus cannot be estimated and locus-specific heritability cannot be obtained using these methods. $ROMP_{rev}$ provides a formally derived estimator of the locus-specific heritability in samples of independent parent-offspring trios with no parental genotypic data and thus provides researchers with the choice of only phenotyping the parents without losing an accurate estimate of locus-specific heritability.

$ROMP_{rev}$ assumes independent observations and is thus best suited for samples of parent-offspring trios. However, in these simulations, the analysis of 50 families of size 3 as 150 trios by repeating the parental phenotypes increased the type I error from about 0.04 to 0.07 for the parametric $ROMP_{rev}$ approach and provided power comparable to the power obtained with 150 independent trios. Locus-specific heritability estimates were close to the simulated values but with reduced coverage of both the parametric and bootstrap confidence intervals. Bootstrap intervals coverage probability stayed higher than that of the parametric approach and was close to 90% on average. Hence, for small nuclear families of 2 or 3 children, the $ROMP_{rev}$ test of association seems fairly robust to the departure from independence, but caution should be used when interpreting the heritability estimates.

A permutation test was also considered to obtain the p-value for the $ROMP_{rev}$ association test. The permutation approach yielded higher power than the parametric $ROMP_{rev}$ test for small locus-specific heritability. A 30 – 40% increase in power was observed for locus-specific heritabilities of less than or equal to 5% when 150 independent trios were analyzed. However, the permutation test was more computationally intensive.

Additional simulations using the same models were performed to briefly compare the $ROMP_{rev}$ method to an ANOVA using only the offspring and to the test of linkage and association introduced by Abecasis et al. (2000), implemented in the QTDT software (data not shown). QTDT uses phenotypes of offspring and genotypes of offspring and parents and utilizes only trios informative for the transmission of alleles. In the absence of population stratification, the power of $ROMP_{rev}$ was similar to that of ANOVA and higher than that of QTDT. However, $ROMP_{rev}$ does not completely correct for potential population stratification bias. Because the $ROMP_{rev}$ association test is, in a sense, adjusting for the parents' phenotypes, the level to which $ROMP_{rev}$ eliminates population stratification bias depends on how well the offspring-parent phenotype correlation captures the underlying genetic background, i.e., it depends on the heritability of the trait. Only with 100% heritability would $ROMP_{rev}$ completely correct for the population stratification bias.

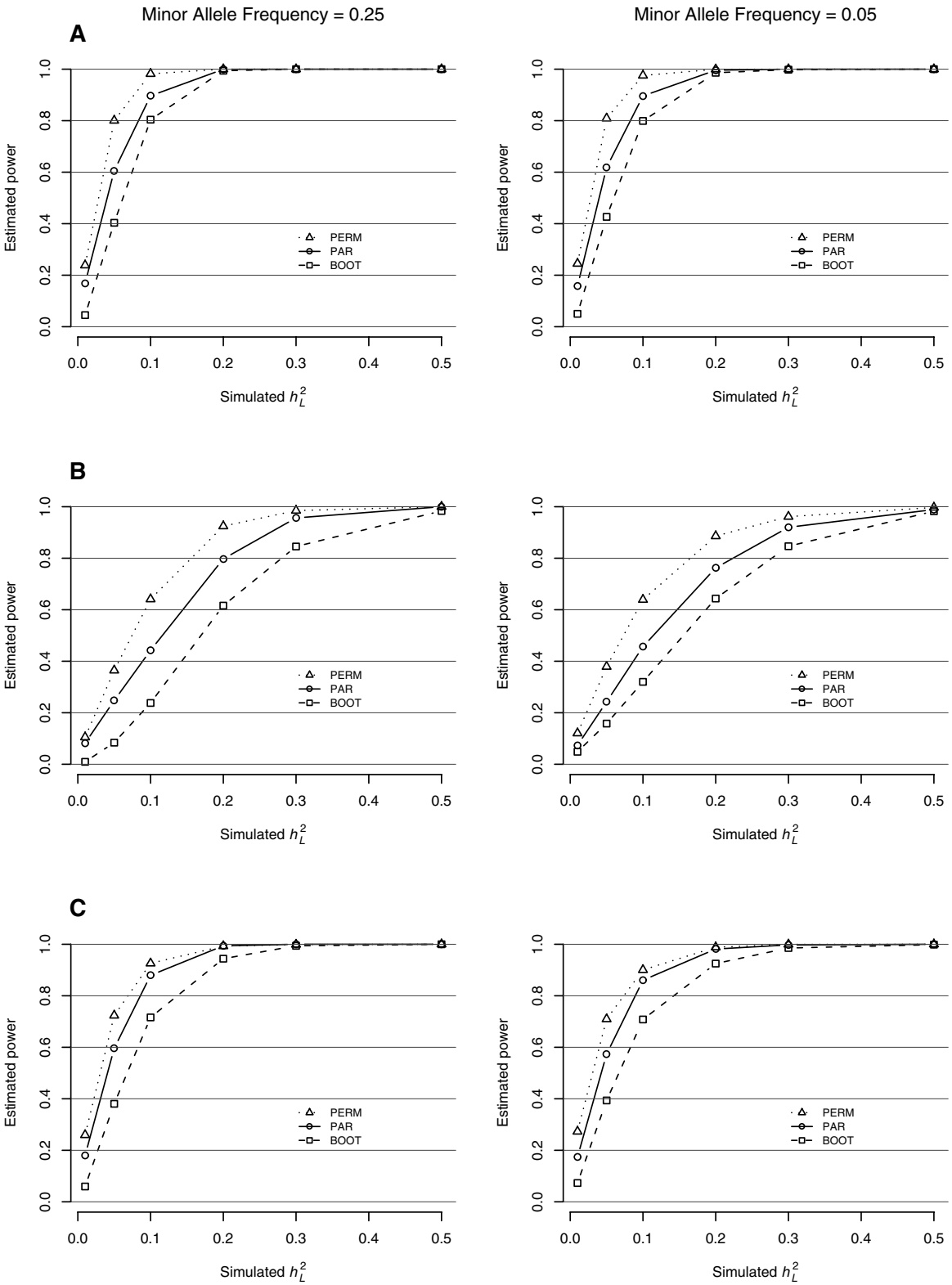

Figure 2 Estimated power at the 0.05 level for the $ROMP_{rev}$ parametric (PAR), bootstrap (BOOT) and permutation test (PERM) approaches in 2000 replicates of A) 150 independent parent-offspring trios, B) 50 independent parent-offspring trios and C) 150 non-independent trios (from 50 nuclear families of size 3).

Table 2 Association between the hemostatic factors and candidate polymorphisms in a sample of independent parent-offspring trios (n = 58): Estimates and p-values from the $ROMP_{rev}$ method, and p-values from FBAT, QTDT, and ASSOC, adjusted for age, gender, smoking and drinking status.

		Estimates and p-values from $ROMP_{rev}$			P-values from:		
Trait ($\widehat{h}^2$)	Polymorphism	$\widehat{h}^2_l \pm$ parametric s.e.	Parametric P-value	Bootstrap 95% confidence interval	FBAT	QTDT	ASSOC
Factor VII (0.65)	Arg353Gln	0.034 ± 0.042	0.4231	(−0.023, 0.119)	0.1525	0.1689	0.1228
Fibrinogen (0.14)	HaeIII	0.038 ± 0.011	0.0012	(−0.088, 0.176)	0.0040	0.0010	0.0023
	AluI	0.038 ± 0.011	0.0012	(−0.085, 0.171)	0.0040	0.0010	0.0023
	MnlI	0.045 ± 0.014	0.0020	(−0.078, 0.185)	0.0036	0.0008	0.0029
PAI −1 (0.47)	4G/5G	0.016 ± 0.012	0.1931	(−0.056, 0.122)	0.1514	0.0814	0.0504

Given that real data is often plagued with missing values, an approach based on the regression of offspring on one parent ($ROOP_{rev}$) can be used as an alternative to $ROMP_{rev}$ when parental phenotype is available on only one parent. $ROOP_{rev}$ provides the same heritability estimates as $ROMP_{rev}$ and in our simulations, the power of the $ROOP_{rev}$ method to detect locus-specific effects was very close to that of $ROMP_{rev}$ (data not shown). In situations where data include a mixture of completely phenotyped trios and some missing parental phenotypes, $ROMP_{rev}$ and $ROOP_{rev}$ may be combined in one estimator.

In this simulation study, the coverage probability of the $ROMP_{rev}$ parametric 95% confidence intervals for the locus-specific heritability was consistently lower than 95% when the simulated locus-specific heritability was greater than zero. However, the parametric test of significance showed appropriate type I error and good power. This apparent disagreement between the reduced coverage of the confidence intervals and the accurate type I error may reflect a different distribution of the statistic when a locus effect is present compared to no locus effect. A similar problem was previously reported in the literature in the context of the study of intervening variables effects or mediation, which is closely related to $ROMP_{rev}$. MacKinnon et al. (2002) argued that the difference in slopes from models (1) and (2) is not expected to be normally distributed, and that an underestimation of the standard error compensates for the use of inappropriate critical values from a normal distribution, hence restoring accurate type I error. In light of this problem, a bootstrap approach to obtain standard errors and confidence intervals was implemented and yielded satisfying coverage. However, the bootstrap confidence intervals were too large under the null hypothesis and the power of a test based on the intervals was low. A two-step approach is suggested. First, a $ROMP_{rev}$ heritability estimate (along with its parametric standard error) combined with the parametric test of association, which showed good properties, should provide a quick result for a large number of SNPs. Once association is established, obtaining a bootstrap confidence interval around the locus-specific heritability estimate seems to be worthwhile to refine the estimation as a second step for polymorphisms of interest. A similar two-step approach for testing and parameter estimation was previously suggested in the context of linkage analysis (Liang et al. 2001).

An interesting application of $ROMP_{rev}$ could be in samples of cases that have already been collected and for which parental phenotype data is available or easy to obtain. $ROMP_{rev}$ would then provide an easy way to test for association and estimate heritability. However, the ideal sample for $ROMP_{rev}$ is a population-based sample of parent-offspring trios and a concern with the sample of cases is selection on the dependent variable in $ROMP_{rev}$: the phenotype of the offspring, i.e., the cases. The effect of selection on $ROMP_{rev}$ is related to the effect of truncation on least-squares regression coefficients (Long, 1997, Chapter 7). The trait heritability will be underestimated if large trait values are selected for, or overestimated if small trait values are selected for. However, a simple way of addressing this problem is to include some trios of controls and their parents with the trios of cases and their parents, in the $ROMP_{rev}$ analysis in order to capture all the variation in the distribution of the offspring trait. The effect of selection on the $ROMP_{rev}$ test of association and locus-specific heritability estimation is less clear and requires further investigation.

An interesting question that was not directly addressed in these simulations is the ability of $ROMP_{rev}$ to detect two or more 'major' loci. Simulations to answer this question were performed for up to 10 loci with the test of association based on the coefficient γ_l of the locus effect in model (2), which is, as discussed above, very similar to the $ROMP_{rev}$ test based on the locus-specific heritability estimate (data not shown and Roy-Gagnon, 2004). For fixed trait heritability, the power of $ROMP_{rev}$ to detect a specific locus effect size did not change when additional major loci were

included in the model instead of a polygenic effect, so that all loci could be detected with the same power. The power was mainly determined by the size of the locus effect and secondly by the total trait heritability whether it was due to polygenes or other major loci.

In the application of the $ROMP_{rev}$ method presented here, the contribution of known candidate polymorphisms to the heritability of coagulation factor VII, fibrinogen and PAI −1 was estimated. ROMP's ability to estimate locus-specific heritability gave an indication of the role played by the candidate polymorphisms in the heritability of the traits. Significant association was found between fibrinogen and three polymorphisms located within the fibrinogen gene cluster. Total heritability for fibrinogen adjusted for age, gender, smoking and drinking status was estimated to be 14%, which is lower than reported estimates from other population (de Lange et al. 2001; Freeman et al. 2002; Souto et al. 2000). As seen in the simulation results, the bootstrap yielded lower power than the parametric approach and given the small sample size the bootstrap confidence intervals for the locus-specific heritability estimate of 4% included 0. Estimates should thus be interpreted with caution. Nonetheless, these results suggest that this locus explains a significant part of the fibrinogen heritability in this Korean sample. The $ROMP_{rev}$ results were corroborated by the FBAT, QTDT and ASSOC results.

In summary, the $ROMP_{rev}$ method provides a test of association between a quantitative trait and a locus, and an unbiased estimate of the locus-specific heritability along with a large-sample and a bootstrap estimate of the variance. A computer program for the $ROMP_{rev}$ method, implemented in the R language (Ihaka & Gentleman, 1996), is available from the National Human Genome Research Institute Online Research Resources webpage (http://research.nhgri.nih.gov/). $ROMP_{rev}$ requires data from independent parent-offspring trios and can be useful to test for association between quantitative traits and a large number of SNPs in a candidate region, a candidate gene, or a whole-genome association study. It can be especially useful when phenotypic data on parents are less costly and/or more readily available than genotypic data, since it requires phenotypes from offspring and parents but genotypes from offspring only. Locus-specific heritability estimates may be particularly useful in studying the etiology of complex traits in which several genes and environmental factors are likely to play a role.

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1.

Appendix S2.

Table S1. Conditional probability of a parental genotype, g_p given the genotype of the offspring equals g_o

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-1809.2007.00401.x
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