

SHORT REPORT

Meta-analysis of SNPs involved in variance heterogeneity using Levene's test for equal variances

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Meta-analysis is a commonly used approach to increase the sample size for genome-wide association searches when individual studies are otherwise underpowered. Here, we present a meta-analysis procedure to estimate the heterogeneity of the quantitative trait variance attributable to genetic variants using Levene's test without needing to exchange individual-level data. The meta-analysis of Levene's test offers the opportunity to combine the considerable sample size of a genome-wide meta-analysis to identify the genetic basis of phenotypic variability and to prioritize single-nucleotide polymorphisms (SNPs) for gene–gene and gene–environment interactions. The use of Levene's test has several advantages, including robustness to departure from the normality assumption, freedom from the influence of the main effects of SNPs, and no assumption of an additive genetic model. We conducted a meta-analysis of the log-transformed body mass index of 5892 individuals and identified a variant with a highly suggestive Levene's test *P*-value of 4.28E-06 near the *NEGR1* locus known to be associated with extreme obesity.

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INTRODUCTION

Considering the effects on the phenotypic variance of quantitative traits (that is, differences in the variance of a trait according to the genotype), a sample size of the order of tens of thousands is required for the association of single-nucleotide polymorphisms (SNPs) at genome-wide significance levels ($P < 1E-06$).¹ The effects on the phenotypic variance of quantitative traits are likely to be equally modest, thus requiring large sample sizes to identify. Among the statistical tests designed to detect variance heterogeneity, Levene's test² has been shown to be robust to the violation of the normality assumption and adequately powered under other irregularities.³ Furthermore, Levene's test, by design, is not under the influence of any of the main effects of SNPs and compares the pairwise differences in variance between genotype groups, which encompass both linear and non-linear trends. For instance, an analysis of 21 799 individuals from the Women's Genome Health Study first identified SNPs with a genome-wide significant Levene's test *P*-value for C-reactive protein (rs12753193, $P = 8.0E-11$) and soluble ICAM-1 (rs738409, $P = 1.9E-10$; rs1799969, $P = 2.1E-09$).⁴ Although it is feasible to analyze the heterogeneity of variance in individually large studies, sufficient sample sizes for the detection of variants with small effects can only be practically reached through meta-analysis. Indeed, a recent report has associated an *FTO* variant (rs7202116) with the phenotypic variability of body mass index (BMI) ($P = 2.4E-10$; $N = 131\,233$) in a meta-analysis using the squared residual as the response variable.⁵

In addition to finding genetic variants influencing phenotypic variance, a meta-analysis of variance heterogeneity can also be used to

prioritize potentially interacting variants to test for gene–environment and gene–gene interactions. The high-dimensional nature of genome-wide data inevitably poses computational and statistical challenges, such as multiple testing burden. Consequently, sample sizes of individual genome-wide association studies have been largely underpowered to detect interactions.⁶ Despite these challenges, there is a pressing need to understand how genetic interactions contribute to the 'missing heritability'.^{7,8} The discovery of novel genetic interactions through meta-analysis presents a promising strategy, as large international consortia provide the adequate sample sizes and methodologies for meta-analyzing interactions are quite well developed.^{9,10} We have previously proposed a prioritization scheme—variance prioritization—in the context of quantitative traits based on the observation that the trait variance conditional on genotypes will vary when an interaction is present,⁴ an active area of methodological research.^{11–13} Prioritization is achieved by comparing the variances of a quantitative trait conditional on the genotypes using Levene's test. As only SNPs with Levene's test *P*-values that are lower than a pre-determined threshold (typically a nominal significance level at ~ 0.05) are tested for interaction effects, the underlying effect of multiple hypothesis testing is greatly reduced and overall statistical power is increased accordingly, compared with an exhaustive search for gene–gene or gene–environment interactions.

In this paper, we provide a framework for combining summary statistics from multiple genome-wide studies to calculate the meta-analyzed Levene's test *P*-values for individual SNPs without needing to exchange individual-level data. We then perform a genome-wide search for SNPs involved in the heterogeneity of variance using log-transformed BMI and height.

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MATERIALS AND METHODS

Consider a quantitative trait Y with N individuals, and Y_i as the quantitative trait when stratified according to the possible genotypes ($i = 0, 1, \text{ or } 2$) of a biallelic SNP. To obtain an equivalent of the exact Levene's test statistic without exchanging individual-level data, the following statistics are reported by the study s ($s = 1, 2, \dots, S$) for each SNP:

(n_{0s}, n_{1s}, n_{2s}) : genotype counts, summing up to N_s
 $(\bar{Z}_{0s}, \bar{Z}_{1s}, \bar{Z}_{2s})$: within genotype means of Z_0, Z_1, Z_2
 $(\sigma_{0s}^2, \sigma_{1s}^2, \sigma_{2s}^2)$: within genotype variances of Z_0, Z_1, Z_2

Where $Z_i = |Y_i - \bar{Y}_i|$ and \bar{Y}_i is the group mean of Y_i . The calculation of Levene's test statistic by simply combining samples assumes the following

natural weights: $\omega_{is} = \frac{n_{is}}{\sum_s n_{is}}$ ($\sum_s \omega_{is} = 1$) and $\gamma_i = \frac{\sum_s n_{is}}{\sum_i \sum_s n_{is}} = \frac{n_i}{N}$ ($\sum_i \gamma_i = 1$).

The meta-analyzed Levene's test statistic L^+ using only the summary statistics and weights is (detailed derivation in S1):

$$L^+ = \frac{(N-3)}{(3-1)} \times \frac{(\sum_{i=0}^2 \gamma_i \times (\sum_s \bar{Z}_{is} \times \omega_{is})^2 - (\sum_{i=0}^2 \sum_s \bar{Z}_{is} \times \omega_{is} \times \gamma_i)^2)}{\sum_{i=0}^2 (\sum_s (\sigma_{is}^2 \times \omega_{is} - \frac{\sigma_{is}^2}{N \times \gamma_i} + \bar{Z}_{is}^2 \times \omega_{is}) \times \gamma_i - ((\sum_s \bar{Z}_{is} \times \omega_{is})^2 \times \gamma_i)}$$

Under the null hypothesis of variance homogeneity, L^+ follows an F -distribution with $df_1 = 2$ and $df_2 = N - 3$. Caution should be observed regarding rare variants (minor allele frequency (MAF) $< 1\%$); a minimum of two individuals is needed to estimate the variance in any observed genotype group.

It is common practice in meta-analysis to apply study-specific weights in such a way that the combined estimate reflects the individual effects of varying influences. An adjusted weight can be attained by multiplying the natural weights by the desired adjustment η_{is} :

$$\omega_{is, \text{adjusted}} = \eta_{is} \times \omega_{is}, 0 \leq \eta_{is} \leq 1$$

$$\gamma_{i, \text{adjusted}} = \frac{\sum_s n_{is} \times \eta_{is}}{\sum_i \sum_s n_{is} \times \eta_{is}}$$

The corresponding P -value can be calculated from the test statistic, with the adjusted weights replacing the natural weights. In other words, natural weights are re-weighted by the adjustment $\eta_{is} \in [0, 1]$, where 1 corresponds to the complete representation of the i th genotype in the s th study and 0 corresponds to no representation in the meta-analysis.

We conducted a genome-wide meta-analysis of the variance heterogeneity for log(BMI) and log(height) using three publicly available genome-wide data sets from dbGap:¹⁴ MESA (Study accession: phs000209.v10.p2, http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000209.v10.p2) and GENEVA, including data from the NHS and HFPS (Study accession: phs000091.v2.p1, http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000091.v2.p1). For each data set, we performed quality control of SNPs based on MAF ($> 1\%$), Hardy-Weinberg equilibrium ($P > 1E-08$) and the genotype call rate ($> 95\%$) and filtered individuals based on ethnicity (only European Caucasians) and relatedness (kinship coefficient > 0.025). In addition, individuals with diabetes were excluded from the analysis of BMI to avoid reverse causation.

RESULTS

The quantile-quantile plots of the Levene's test P -values suggested no noticeable inflation of type I error rate in either the individual studies or the meta-analysis (Supplementary Figure S2). We did not detect SNPs with a meta-analyzed Levene's test P -value (as shown in Figures 1a and e) that was lower than $5E-08$ (ref. 15), which was attributed to the small sample size, even with all the studies combined (Supplementary Table S1). For SNPs with P -values that were lower than $1E-05$ (Table 1), we systematically searched for neighboring SNPs associated at genome-wide significance levels with any traits or disease in the catalog of published GWAS (<http://www.genome.gov/gwastudies/>), filtering associations based on a maximum distance of 500 kb and $r^2 > 0.8$ or $D' > 0.8$. Among the 16 top hits for log(BMI), rs12132044 in the intronic region of the *NEGR1* gene had a highly suggestive meta-analyzed Levene's test P -value of $4.28E-06$ (Supplementary Figure S3). Notably, rs2815752 near the *NEGR1*

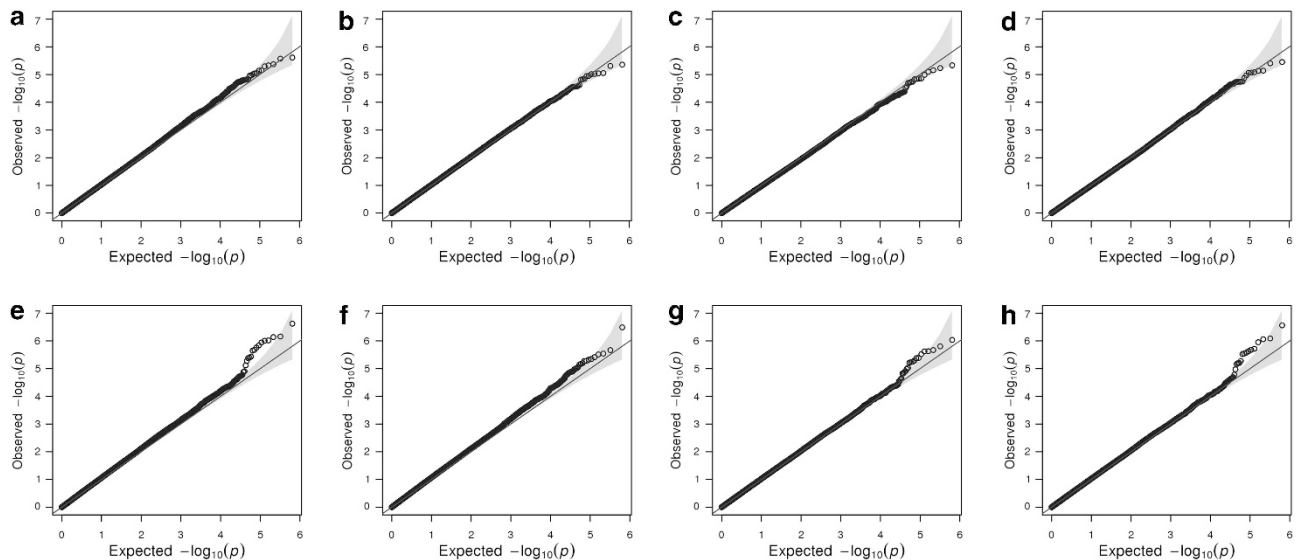


Figure 1 Distribution of meta-analyzed Levene's test P -values according to study weights for log(height) and log(BMI). Illustrated in (a–d) is the quantile-quantile plot of meta-analyzed Levene's test P -values for log (height) with adjusted weights applied to the three studies (8114 individuals combined). Illustrated in (e–h) is the quantile-quantile plot of meta-analyzed Levene's test P -values for log (BMI) with adjusted weights applied to the three studies (5892 individuals combined). Panels a and e assumed natural weights, that is, an adjustment of one for all three studies (equivalent to using all the samples from the three studies). For illustrative purposes, we also performed meta-analysis using arbitrary adjustments. Panels b and f assumed an adjustment of 1, 1, and 0 for MESA, NHS, and HPFS, respectively (equivalent to meta-analysis of only MESA and NHS). Panels c and g assumed adjustment of 1, 1, and 0.8 for MESA, NHS, and HPFS, respectively. Panels d and h assumed adjustment of 1, 0.9, and 1 for MESA, NHS, and HPFS, respectively.

Table 1 SNPs with Levene's test P -value lower than 1.0×10^{-5} from a meta-analysis of height and body mass index

SNP	Chr	Position (Kb)	Variance per 0, 1, and 2 minor alleles (MESA)			Variance per 0, 1, and 2 minor alleles (NHS)			Variance per 0, 1, and 2 minor alleles (HPFS)			Nearest gene	N_0	N_1	N_2	Meta P -value
			0	1	2	0	1	2	0	1	2					
BMI																
rs11206673	1	56098928	1.053	0.869	0.872	0.924	0.834	0.815	0.976	0.751	0.638	LOC100507652	3414	1555	159	2.45E-07
rs12058087	1	56091845	1.049	0.873	0.893	0.929	0.838	0.8	0.978	0.741	0.679	LOC100507652	3497	1531	166	7.11E-07
rs10493187	1	56093557	1.048	0.874	0.872	0.929	0.837	0.798	0.974	0.75	0.679	LOC100507652	3500	1527	169	7.45E-07
rs12118218	1	56093033	1.049	0.872	0.872	0.926	0.838	0.8	0.974	0.752	0.681	LOC100507652	3494	1534	167	9.87E-07
rs3851114	11	56923237	0.973	0.938	1.183	0.953	0.819	0.998	0.938	0.817	1.043	PRG2	1728	2549	919	1.02E-06
rs549630	11	56925282	0.983	0.934	1.191	0.933	0.824	1.003	0.937	0.816	1.083	PRG2	1960	2421	720	1.17E-06
rs6036589	20	23886338	1.014	0.914	1.225	0.933	0.801	1.145	0.943	0.781	1.188	GGTLC1	2968	1854	288	1.50E-06
rs4573554	1	56170625	1.067	0.892	0.884	0.949	0.837	0.793	0.988	0.768	0.92	LOC100507652	2878	1979	335	1.78E-06
rs4468206	1	56169950	1.066	0.891	0.928	0.95	0.837	0.793	0.989	0.768	0.92	LOC100507652	2880	1978	336	2.16E-06
rs12123418	1	56155982	1.042	0.888	0.83	0.94	0.832	0.782	0.955	0.764	0.592	LOC100507652	3506	1488	144	2.29E-06
rs555097	11	56919292	0.989	0.931	1.187	0.935	0.833	1.007	0.93	0.82	1.072	PRG2	2017	2449	730	3.78E-06
rs12119831	1	56164598	1.06	0.896	0.945	0.948	0.837	0.8	0.986	0.767	0.92	LOC100507652	2912	1953	330	4.10E-06
rs12132044	1	72306264	0.854	1.058	1.042	0.792	0.936	0.974	0.803	0.944	0.939	NEGR1	1647	2600	944	4.28E-06
rs6670350	1	56164394	1.057	0.895	0.947	0.948	0.838	0.8	0.986	0.767	0.92	LOC100507652	2912	1952	329	5.38E-06
rs3850552	1	57638259	1.14	0.887	1	1.009	0.845	0.829	1.005	0.9	0.699	DAB1	1755	2569	872	7.79E-06
Height																
rs11224301	11	99959951	1.013	0.894	1.508	1.005	0.909	1.782	1.011	0.854	1.727	ARHGAP42	6857	1161	52	2.48E-06
rs10014562	4	168855855	1.068	0.906	1.099	0.986	0.954	1.234	1.011	0.901	1.231	ANXA10	3626	3582	869	2.70E-06
rs12919408	16	13831900	0.931	1.18	1.153	0.957	1.089	1.326	0.959	1.065	1.132	ERCC4	6005	1921	177	4.29E-06
rs2182965	10	130314001	0.951	1.092	0.973	0.942	1.092	1.123	0.931	1.091	0.993	MKI67	5044	2583	350	4.74E-06
rs7153476	14	68102983	0.973	1.05	0.906	0.915	1.044	0.981	0.917	1.056	0.916	RAD51L1	2163	4107	1837	5.12E-06
rs857179	16	13838131	0.928	1.186	1.155	0.962	1.078	1.261	0.959	1.061	1.169	ERCC4	5979	1942	182	7.09E-06
rs427118	6	92400922	1.038	0.928	1.124	0.997	0.96	1.107	0.981	0.93	1.199	MIR4643	3117	3809	1176	7.37E-06
rs16893439	4	15999108	0.937	1.082	0.965	0.946	1.074	0.913	0.919	1.041	1.256	LDB2	4416	3153	537	8.97E-06
rs1523354	3	20543327	1.037	1.009	0.802	1.085	0.949	0.851	1.063	0.928	0.933	SGOL1	3603	3540	962	9.26E-06
rs10769948	11	8718358	1.019	0.936	1.107	1.046	0.957	1	1.04	0.867	1.252	ST5	2519	3966	1528	9.93E-06

Abbreviations: BMI, body mass index; SNP, single-nucleotide polymorphism.

Minor allele frequency and Levene's test P -value estimated from individual studies were recorded along with the combined Levene's test P -value (meta-analyzed P -value) and pooled genotype counts. N_0 , N_1 , and N_2 represent the pooled genotype counts in the major allele homozygote, heterozygote, and minor allele homozygote group, respectively.

gene, which is known to be associated with BMI¹⁶ and severe obesity in a pediatric cohort,⁵ was in weak LD ($r^2=0.325$; $D'=0.888$; Distance = 231.44 kb) with rs12132044 and also nominally significant for variance heterogeneity ($P=0.0076$). None of the other top hits for log(BMI) or log(height) were correlated with variants associated with other traits or diseases in their neighboring regions (Supplementary Table S2). For illustrative purposes, we also performed a meta-analysis using arbitrary adjustments. Similar conclusions were reached when meta-analysis was performed with study-specific weights (Figures 1b–d, f and g). Additional simulation results on variance prioritization are provided (Supplementary Table S3; Supplementary Figure S1 and S4).

DISCUSSION

Analysis of the genetic basis of quantitative trait variance has recently gained increasing interest. Differences in the variance of a quantitative trait between genotypes of a SNP can be due to environmental sensitivity, underlying gene–gene or gene–environment interactions, or linkage disequilibrium with causal variants. Levene's test can be applied to meta-analysis of environmental sensitivity, which largely rests on analysis of phenotypic variation. Notably, meta-Levene identified a NEGR1 variant (rs12132044) such that the variance of log(BMI) stratified by genotypes was related to its number of minor alleles in a non-linear fashion, which would otherwise be underpowered for detection using a linear model.

Even with the large sample size available from modern consortia, statistical power to detect interactions remains modest, and thus there is a need for prioritization methods. The computation of Levene's test P -values using only summary-level data facilitates the use of variance prioritization in meta-analysis when individual-level data cannot be obtained. In variance prioritization, SNPs with significant Levene's test P -values are prioritized and then directly tested for interaction effects using the preferred interaction meta-analysis methods. Our simulations (Supplementary Table S3) showed improvements in power when using the optimal Levene's test P -value thresholds. Increased power was consistent with the reductions in the genetic interaction search space resulting from the prioritization of SNPs. Under most circumstances, the absolute power to detect an interaction is low, *a priori*, such that the relative increase in power is substantial. This can be highly advantageous if hundreds or thousands of interactions of small effect sizes underlie the genetics of complex traits. On the other hand, the need for prioritization diminishes when the interaction effect sizes are large and exhaustive search alone provides satisfactory power. However, even in this scenario, the performance of prioritization is either better than or at least equivalent to the conventional exhaustive search. Finally, the strength of association between the environmental covariate and the quantitative trait is the main determinant of the gain in power from prioritization, so situations where variance prioritization is particularly favorable can be readily identified. Our simulations (Supplementary Figure S4) also concurred with the theoretical

framework of variance prioritization^{4,11}, according to which the sample size, number of SNPs, MAF, and the proportion of variance explained by the interactions and the covariate influenced the statistical power of variance prioritization.

Beyond allowing the implementation of variance prioritization to select SNPs for a meta-analysis of genetic interactions or environmental sensitivity, there are many other potential applications of the meta-analysis of Levene's test. For example, the homogeneity of variance assumption underlying many statistical models is usually examined using Levene's test. The meta-analysis of the main effects often relies on the assumption of a common variance among the different levels of a factor, and meta-analysis of Levene's test can be conveniently adopted as a quality control step prior to main effects analysis. Meta-analysis of Levene's test is not limited to stratification by genotype; it can also be used to investigate the heterogeneity of phenotypic variance across a wide range of environmental factors.

A few limitations are worth considering. First, the required summary statistics are not typically reported in existing GWAS meta-analyses, and the generation of such statistics entails further analytic efforts among individual research centers. However, calculation of summary statistics can be simply executed at research centers using our PLINK R plug-in¹⁷ scripts (PLINK v.1.07; <http://pngu.mgh.harvard.edu/purcell/plink/>). Second, meta-analysis frequently uses imputation methods to produce a common set of SNPs among studies genotyped on different platforms. Imputed SNPs are usually assigned a probability score based on the expected number of minor alleles, in which case individuals cannot be stratified into discrete genotypes. To address this concern, we suggest using a best-guess model whereby participants may be classified according to the most likely genotype. However, further statistical methodologies to incorporate probabilistic genotypes under the current framework are required. Finally, population stratification presents a major challenge to the meta-analysis of population-based GWAS. We observed that meta-analysis of Levene's test in a multi-ethnic population can lead to false positive results when no precaution is taken (data not shown). Although our method does not explicitly address this problem, one solution would be to compute the required summary statistics from the principal-component-adjusted traits.

In conclusion, we have presented a mathematical framework for meta-analysis of Levene's test that can be used for environmental sensitivity or variance prioritization in meta-analysis. The use of Levene's test is advantageous as it is robust to departures from the

normality assumption, is not influenced by the main effects of SNPs, and does not assume an additive genetic model. Finally, meta-analysis of Levene's test can be adapted to more general contexts of variance analysis and has utility beyond the field of genetics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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