Exploration and comparison of methods for combining population- and family-based genetic association using the Genetic Analysis Workshop 17 mini-exome

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Exploration and comparison of methods for combining population- and family-based genetic association using the Genetic Analysis Workshop 17 mini-exome

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Abstract
We examine the performance of various methods for combining family- and population-based genetic association data. Several approaches have been proposed for situations in which information is collected from both a subset of unrelated subjects and a subset of family members. Analyzing these samples separately is known to be inefficient, and it is important to determine the scenarios for which differing methods perform well. Others have investigated this question; however, no extensive simulations have been conducted, nor have these methods been applied to mini-exome-style data such as that provided by Genetic Analysis Workshop 17. We quantify the empirical power and false-positive rates for three existing methods applied to the Genetic Analysis Workshop 17 mini-exome data and compare relative performance. We use knowledge of the underlying data simulation model to make these assessments.

Background
Study designs for genetic association studies fall into two broad categories: (1) population-based studies that recruit unrelated individuals and (2) family-based studies that collect some number of related pedigrees. Often, both study designs are used for a particular investigation. For example, when a linkage study has been performed and family data are collected, follow-up analysis can include association using a new unrelated study population. The analytic methods appropriate for either design differ, thus making difficult the aggregation of the association metrics across the study designs. Heuristically, population-based metrics attempt to quantify a measure of correlation or association between some function of genotype at a given marker and the disease phenotype, whereas family-based association measures use properties of Mendelian transmissions from parents to offspring and are inherently conditional.

Because analyzing the disparate types of data in isolation most often results in nonoptimal statistical power, investigators have proposed several methods for efficiently combining these data. We briefly summarize three methods to be applied to the Genetic Analysis Workshop 17 (GAW17) data in the Methods section. Each approach is distinguished by the study designs for which it is appropriate, the assumptions necessary for valid inference, and the handling of population stratification (whether it is formally or informally tested or whether it is taken into account by means of adjustments). Operationally, these methods are distinguishable by computation and implementation considerations and by empirical performance. We assess the performance in this paper. Other researchers have investigated the question of relative performance [1]; however, no simulations have been conducted for comparison.

An important consideration to keep in mind throughout this investigation is the underlying causal model that was used to generate the GAW17 data [2]. First, rather than reflecting the common disease/common variant hypothesis that the established methods presented...
address, the data-generating mechanism used was consistent with the multiple rare variant or the common disease/rare variant (CDRV) hypothesis, which suggests that common disease susceptibility is garnered through multiple rare variants with moderate to high penetrance. Intuitively, the current methods do not perform well in identifying rare single-nucleotide polymorphisms (SNPs); in this paper we intend to assess this performance and to motivate possible modifications that would be successful when the CDRV hypothesis is true. In addition, the disease was simulated to have \( \geq 30\% \) prevalence, which violates the often-invoked rare disease assumption.

**Methods**

The first attempts to combine population- and family-based association data were developed by Nagelkerke et al. [3], who used a likelihood framework to combine case-control data with family data by exploiting the likelihood formulation [4] of the transmission disequilibrium test (TDT) [5]. This approach assumes Hardy-Weinberg equilibrium (HWE), random mating, and a multiplicative model of allelic effect. Although no formal test of the appropriateness of combining the two types of data has been developed, we discuss ad hoc procedures.

Epstein et al. [6] generalized this work by relaxing the assumptions of HWE, random mating, and the assumed multiplicative mode of inheritance. In addition, they described a formal test for the appropriateness of combining case-control and case-trio data by comparing genotype relative risk (RR) estimates from between-individual and within-family analyses, respectively. The proposed two-stage procedure facilitates valid model selection in the presence of population stratification. Further extensions of this approach were made by Chen and Lin [7]. Their method uses weighted least squares to aggregate families and case-control samples. An estimate for the RR is obtained from the CPG likelihood and is denoted \( \hat{\beta}_{\text{trio}} \). This estimate is then compared to a traditional logistic regression estimate of the genotype log odds ratio, \( \hat{\beta}_{\text{CC}} \), using the case-control sample, which is composed of case-trio probands and the unrelated control subjects. Chen and Lin use a Wald-type test to determine whether the effect estimates are consistent. If this test is not rejected, a weighted least-squares estimator for the combined genetic effect is then constructed for inference as:

\[
\hat{\beta} = W_1 \hat{\beta}_{\text{trio}} + W_2 \hat{\beta}_{\text{CC}}
\]

where \( W_1 \) and \( W_2 \) are weights derived from linear model theory assuming the parameter estimates follow a multivariate normal distribution (see Chen and Lin [7] for details). Here, the assumptions of a rare disease and no population stratification are necessary for validity. However, the test used to reject the appropriateness of combining the RR estimates is not well powered, as evidenced by our simulations, which often did not confer sufficient evidence to reject the null hypothesis of parameter equivalence even though the simulated disease is not, in fact, rare—a necessary condition for such equivalence. This method was designed for case-trio and unrelated control subjects; however, in our analyses control offspring from the control trios are added to the case-control subsample.

**Chen and Lin’s method**

Chen and Lin’s [7] approach uses the conditional on parental genotypes (CPG) approach of Schaid and Sommer [22] to construct the likelihood of the case-trio samples. An estimate for the RR is obtained from the CPG likelihood and is denoted \( \hat{\beta}_{\text{trio}} \). This estimate is then compared to a traditional logistic regression estimate of the genotype log odds ratio, \( \hat{\beta}_{\text{CC}} \), using the case-control sample, which is composed of case-trio probands and the unrelated control subjects. Chen and Lin use a Wald-type test to determine whether the effect estimates are consistent. If this test is not rejected, a weighted least-squares estimator for the combined genetic effect is then constructed for inference as:

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**Zhu et al.’s method**

In Zhu et al.’s [8] approach, principal components are calculated from the genotypes of all unrelated individuals (trio parents and unrelated case and control subjects), and both the genotypes and the phenotypes of these individuals are then separately regressed on the principal components. The resulting linear regression parameter estimates are used to calculate genotypic and phenotypic residuals, \( \hat{y}_i \) and \( \hat{z}_j \), respectively, where \( i \) indexes families and \( j \) indexes individuals within a family. The covariance between these residuals is measured as:
where $N$ is the number of families, $k_i$ is the number of individuals in the $i$th family, and $N_T$ is the total number of individuals. Within-family correlations are taken into account in the calculation of the variance of $T$ to construct a Wald test. Although this method requires enough markers to estimate principal components, it has the distinct advantage of being robust to population stratification. It can incorporate more complex family structures and does not discard any of the GAW17 data for analysis. Software to apply this approach, FamCC, is available from Zhu et al. [8].

Zhang et al.'s method
Zhang et al.'s [9] method adapts a score test statistic proposed by Lange et al. [23] that applies generalized estimating equations to family-based association tests. To obtain estimates for the score test statistic, the components of the test statistic are decomposed into two mutually exclusive sets: the unrelated individuals and the trios. Traits are treated as constants so that the population genotype mean and variance are estimated for the unrelated individuals and the genotype mean and variance for the offspring are defined through Mendelian transmissions. Similar to Zhu et al.’s method, this framework allows for incorporation of covariates, but unlike the other methods considered, it can easily handle missing parents.

Zhang et al. [9] use principal components analysis (PCA) to adjust for population stratification. This is done separately for the two data subsets. The standard principal-components-based adjustment is used for the unrelated individuals and the genotype mean and variance for the offspring are defined through Mendelian transmissions. Although error rate inflation does not appear to be a problem, it is easy to see that all methods are low powered and they handle the changes appear to have a discernible increase in the rejection rates from the null SNPs to the causal SNPs. It also appears that removing so-called spurious genes [25] from the noncausal SNPs lowers the error rate, as expected.

SNP discovery power
Although the power averaged over causal SNPs was low, some of the SNPs were detectable at high rates. Figure 1 displays the empirical powers for each method plotted against the effect size and grouped into three categories of SNP minor allele frequency. Here, effect size is not

### Table 1 Average empirical rejection rates

| Method      | Noncausal SNPs | Causal SNPs |
|-------------|----------------|-------------|
|             | All            | With SNPs from spurious genes removed | All |
| Chen and Lin| 0.0388         | 0.0378      | 0.0269 |
| Zhang et al.| 0.0420         | 0.0408      | 0.0761 |
| Zhu et al.  | 0.0551         | 0.0551      | 0.0556 |

Empirical rejection rates over the 200 replications for each method averaged over all SNPs (24,327 noncausal SNPs and 160 causal SNPs, 2 of which confer susceptibility through two different components of the latent disease susceptibility distribution). Removing SNPs from the spurious genes [25] results in 16,380 noncausal SNPs.
directly for disease status but rather for an underlying distribution of disease susceptibility [2]. It is clear that many rare SNPs are not detectable for any of the examined methods. However, contrary to intuition, many of the rarer SNPs provide the highest levels of power. Those SNPs with substantive power vary between small and large effect sizes. Examining SNPs for which there is at least modest power (Table 2) reveals that the Zhang et al. [9] approach most often is the highest powered.

**Table 2 Empirical rejection rates for top causal SNPs**

| Causal SNP | Gene   | Effect size (β from Simulation Model) | MAF       | Chen and Lin | Zhang et al. | Zhu et al. |
|------------|--------|--------------------------------------|-----------|--------------|--------------|------------|
| C153181    | ELAVL4 | 0.30946                              | 0.000717  | 0            | 0.235        | 0          |
| C153181    | ELAVL4 | 0.76911                              | 0.000717  | 0            | 0.235        | 0          |
| C159189    | PKDC2B | 0.19102                              | 0.006456  | 0            | 0.395        | 0.380      |
| C354880    | BCHE   | 0.20651                              | 0.001435  | 0            | 0.005        | 0.275      |
| C451873    | KDR    | 0.58301                              | 0.000717  | 0.300        | 0.420        | 0          |
| C451878    | KDR    | 0.13573                              | 0.164993  | 0            | 0.305        | 0.120      |
| C454935    | VEGFC  | 1.35726                              | 0.000717  | 0.045        | 0.805        | 0.690      |
| C55153     | FLT4   | 0.15986                              | 0.001435  | 0            | 0.275        | 0          |
| C652981    | VEGFA  | 1.20645                              | 0.002152  | 0.195        | 0.935        | 0.825      |
| C665380    | VNN1   | 0.24437                              | 0.170732  | 0            | 0.580        | 0.450      |
| C85442     | LPL    | 0.49459                              | 0.015782  | 0.050        | 0.280        | 0.260      |
Discussion and conclusions

Several methods address the problem of combining population- and family-based genetic association data. These methods differ fundamentally in whether they incorporate within-family transmissions and rely on tests for population stratification to justify effect estimate aggregation or perform between-individual analyses using family data. Performance related to population stratification cannot be assessed here because no stratification was simulated in the GAW17 data.

Although the Zhang et al. [9] method performed better than the other two methods considered, we did see that no method was well powered to detect causal SNPs in this scenario. Both the Zhang et al. [9] and the Zhu et al. [8] methods allow for more general pedigree structures than the trios-only analysis performed here and will likely perform more favorably when larger pedigrees are considered. In future work, we plan to adapt aggregation methods suitable for the CDRV hypothesis.

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Table 2 Empirical rejection rates for top causal SNPs (Continued)

| SNP   | Method  | Gene  | MAF  | Power | 20%  | 40%  | 60%  |
|-------|---------|-------|------|-------|------|------|------|
| C95444| VLDLR   | 0.86528 | 0.001435 | 0.020 | 0.430 | 0.195 |
| C1053050| SIRT1  | 0.97060 | 0.002152 | 0.025 | 0.215 | 0.040 |
| C1053109| SIRT1  | 0.51421 | 0.000717 | 0     | 0.730 | 0.640 |
| C135431| FLT1    | 0.74136 | 0.001721 | 0.075 | 0.385 | 0.000 |
| C135522| FLT1    | 0.61830 | 0.027977 | 0     | 0.550 | 0.400 |
| C135523| FLT1    | 0.64997 | 0.066714 | 0.055 | 0.685 | 0.660 |
| C1451382| SOS2   | 0.28058 | 0.003587 | 0     | 0.230 | 0.015 |
| C1751043| SREBF1 | 0.49941 | 0.004304 | 0     | 0.270 | 0.155 |
| C1751046| SREBF1 | 0.62779 | 0.002869 | 0     | 0.215 | 0.350 |
| C1751048| SREBF1 | 0.28739 | 0.001435 | 0     | 0     | 0.245 |
| C1754578| PRKCA  | 0.17038 | 0.166428 | 0.010 | 0.355 | 0.520 |

Gene, effect size, minor allele frequency (MAF), and empirical rejection rate over the 200 replications from each method for the 21 causal SNPs conferring ≥20% empirical rejection rate from at least one of the three methods. The maximum empirical rejection rate over the three methods is in boldface for each causal SNP. There are 160 causal SNPs, 2 of which confer susceptibility through two different components of the latent disease susceptibility distribution.

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