A Hybrid Likelihood Model for Sequence-Based Disease Association Studies

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Abstract

In the past few years, case-control studies of common diseases have shifted their focus from single genes to whole exomes. New sequencing technologies now routinely detect hundreds of thousands of sequence variants in a single study, many of which are rare or even novel. The limitation of classical single-marker association analysis for rare variants has been a challenge in such studies. A new generation of statistical methods for case-control association studies has been developed to meet this challenge. A common approach to association analysis of rare variants is the burden-style collapsing methods to combine rare variant data within individuals across or within genes. Here, we propose a new hybrid likelihood model that combines a burden test with a test of the position distribution of variants. In extensive simulations and on empirical data from the Dallas Heart Study, the new model demonstrates consistently good power, in particular when applied to a gene set (e.g., multiple candidate genes with shared biological function or pathway), when rare variants cluster in key functional regions of a gene, and when protective variants are present. When applied to data from an ongoing sequencing study of bipolar disorder (191 cases, 107 controls), the model identifies seven gene sets with nominal p-values < 0.05, of which one MAPK signaling pathway (KEGG) reaches trend-level significance after correcting for multiple testing.

Introduction

Research efforts over the past few years have yielded an explosion of exome sequencing studies and exomic variation data (reviewed in [1,2]). One surprising result has been the discovery of hundreds of thousands of novel and rare nonsilent variants in protein coding genes, some of which may have functional consequences related to human health. Common diseases, once hypothesized to be primarily due to common variants [3], are now believed to have heterogeneous genetic causes, due to both common and rare variants [4–6].

These developments have created demand for a new generation of statistical and informatics approaches. Increasingly powerful analysis methods have been developed to enable detection of association between phenotype and variants with small to moderate effect sizes (reviewed in [6]). Rather than testing each variant individually, variants can be collapsed or summed with a “burden” approach, in which the strength of phenotypic association is considered with respect to a group of variants occurring at a common region or allelic frequency threshold [7–10]. The contribution of each variant to the association may be weighted by frequency or bioinformatically predicted impact [10]. Burden strategies yield a power gain, compared to independent tests of single variants, but they lose power when variants with a neutral or protective effect are included. Regression models [11] and overdispersion tests [12] have been designed to detect variants that affect phenotype, regardless of the direction of the effect (deleterious or protective). New approaches continue to be introduced, such as a mixture model that incorporates gene-gene interactions and an adaptive weighting procedure [13]. A recent study has even suggested that single-variant test statistics may be more powerful than collapsing strategies on real data [14]. Importantly, no single method appears to be superior for all phenotypes, genomic regions, disease models, and populations [6,15,16].

Here we describe a new hybrid likelihood test BOMP (Burden Or Mutation Position test), designed for case-control exome sequencing studies, to detect the presence of causal variants in a functional group. The functional group can be defined as a gene, genomic region, or gene set (multiple genes involved in a pathway or biological process). The test can incorporate variant weighting by bioinformatically-predicted functional impact. We combine, into a single statistic, a directional burden test in which low frequency variants have increased weight and a non-directional position distribution test that does not consider allele frequency. Our burden test uses a collapsing strategy and metrics of variant
Inexpensive, high-throughput sequencing has transformed the field of case-control association studies. For the first time, it may be possible to identify the genetic underpinnings of complex diseases, by sequencing the DNA of hundreds (even thousands) of cases and controls and comparing patterns of DNA sequence variation. However, complex diseases are likely to be caused by many variants, some of which are very rare. Taken one at a time, the association between variant and disease phenotype may not be detectable by current statistical methods. One strategy is to identify regions where important variants occur by “collapsing” variants into groups. Here, we present a new collapsing approach, capable of detecting subtle genetic differences between cases and controls. We show, in extensive simulations and using a benchmark set of genes involved in human triglyceride levels, that the approach is potentially more powerful than existing methods. We apply the new method to an ongoing sequencing study of bipolar cases and controls and identify a set of genes found in neuronal synapses, which may be implicated in bipolar disorder.

Results

We evaluated the power of the BOMP hybrid likelihood model with both simulations and empirical data from the Dallas Heart Study [17]. All results were compared to several leading statistical methods to detect causal variation in case-control association studies. We attempted to select representative methods for burden, regression, and mixture modeling approaches.

First, we assessed the power of BOMP to detect genes with causal variants in an extreme phenotype case-control study, for a disease with 1% population prevalence, and significance level $\alpha = 0.05$. We considered that deleterious causal variants might either be rare, low frequency or common and that modifying protective variants might be present. Power to detect causal variants was assessed initially with respect to a single candidate gene and then for candidate gene sets, ranging in size from 2 to 24 genes. We studied gene sets in which all genes contained causal variants and those in which only a fraction of genes contained causal variants. Both African-American and European-American demographic models were considered. For each combination of attributes (disease etiology, population demographic, case-control study size), 250 case-control studies were simulated to assess power.

Power analysis of simulated case-control studies

In single-gene case-control study simulations, a study size of 2000 (1000 cases, 1000 controls) was required for any of the methods to achieve at least 80% power to detect causal variants. BOMP had >80% power for three of the tested disease etiologies (Common variant, KeyRegion+Protect, and Common+Protect). When the study size was increased to 5000 (or 10000 (Figure S1)), several of the methods (BOMP, SKAT, VT, and KBAC5P (MAF <5%) had >80% power for selected etiologies (Figure 1). BOMP was consistently more powerful than other methods and appeared to be particularly useful for certain disease etiologies (Key region variant, Common variant, and all etiologies involving protective variants (Table 1)). All methods were less powerful when applied to case-control studies using the European-American demographic model (in which variants are either rare or singletons) (Figure S2).

Next, we explored how the power of the tested methods could be improved by application to a candidate gene set rather than a single candidate gene. We simulated case-control studies, in which each genomic individual had multiple genes, all or some of which contained causal variants. The gene sets in which all genes contained causal variants ranged from 2 to 5 genes. Gene sets with mixtures of causal and non-causal genes ranged from 4 to 15 genes (ratios of causal to non-causal 3:1, 3:3, 3:6, 3:9, and 3:12). Causal variants were equally likely to be from any of the disease etiologies dominated by rare variants. The assumption that even 25% of genes in a set contain causal variants is certainly optimistic, but this experiment allowed us to compare the extent to which each method was affected by the fraction of causal genes in a set.

When all genes in a gene set contained causal variants, power increased for all methods as gene set size increased. When the gene sets contained a mixture of genes, both with and without causal variants, the power decreased with the causal to non-causal ratio. For the African-American demographic model, BOMP and SKAT were the most robust to gene sets with low causal to non-causal ratio. As in the single gene experiment, all methods had less power in the European-American demographic than in the African-American. For the European-American, none of the methods had power >80% for any of the gene sets. For the African-American demographic, BOMP, SKAT, and VT had power >80% when gene sets of sizes 4 and 5 contained all causal variants. BOMP was the only method with power > 80% for any of the mixed gene sets tested (gene set sizes 3,6, and 9, with ratio of causal to non-causal 3:0, 3:3 and 3:6) (Figure 2).
Next, we reconsidered the assumption that casual variants in a gene set were equally likely to come from a few disease etiologies. Instead, we sampled disease etiologies from nine multinomial distributions (Figure S3). For these experiments, the number of candidate genes was fixed at nine and the ratio of causal to non-causal genes was 3:6. BOMP’s power advantage over the other tested methods was larger in this experiment than in the single candidate gene experiment. For a case-control study size of 1000, BOMP power was >80% regardless of the causal-to-non-causal gene ratio (African-American only), and for the 1:1 and 3:1 ratios for European-American. A study size of 200 was sufficient for power >0.8 for 1:1 and 3:1 ratios (African-American only) (Figure 4).

We explored the power of BOMP with respect to case-control study size, using a set of 24 candidate genes as the functional group. We varied the ratio of causal to non-causal genes from 1:3, 1:1, and 3:1. Here, causal variants were again equally likely to be from any of the disease etiologies dominated by rare variants. For a case-control study size of 1000, BOMP’s power exceeded 0.8, regardless of the causal-to-non-causal gene ratio (African-American only), and for the 1:1 and 3:1 causal-to-non-causal gene ratios for European-American. A study size of 200 was sufficient for power >0.8 for 1:1 and 3:1 ratios (African-American only) (Figure 4).

We reasoned from these results that, for a population whose allele frequency spectrum is similar to our European-American demographic model simulations, current whole-exome case-control studies are not sufficiently powered. These studies lack power to find causal variants both at the single gene level (as proposed by [19]) and for modestly-sized gene sets. However, if
**Table 1.** Eight disease etiologies used in simulation experiments.

<table>
<thead>
<tr>
<th>Disease Etiology Name</th>
<th>MAF Deleterious</th>
<th>Selection Coeff Deleterious</th>
<th>Effect size Deleterious</th>
<th>Selection Coeff Protective</th>
<th>Effect size Protective</th>
<th>Variant Functional Role</th>
<th>Demographic model(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare variant</td>
<td>≤1%</td>
<td>(−1, −0.0001]</td>
<td>0.5σ</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
<td>AA,EA</td>
</tr>
<tr>
<td>Low frequency variant</td>
<td>(0.1%, 5%)</td>
<td>(−1, −0.0001]</td>
<td>0.5σ</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
<td>AA,EA</td>
</tr>
<tr>
<td>Key region variant</td>
<td>≤1%</td>
<td>(−1, 0)</td>
<td>1.0σ</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
<td>AA,EA</td>
</tr>
<tr>
<td>Common variant</td>
<td>&gt;5%</td>
<td>(−1, 0)</td>
<td>0.1σ</td>
<td>NA</td>
<td>NA</td>
<td>NS</td>
<td>AA</td>
</tr>
<tr>
<td>Rare+Protect</td>
<td>≤1%</td>
<td>(−1, −0.0001]</td>
<td>0.5σ</td>
<td>&gt;0</td>
<td>−0.5σ/−0.1σ</td>
<td>NS</td>
<td>AA</td>
</tr>
<tr>
<td>LowFreq+Protect</td>
<td>(0.1%, 5%)</td>
<td>(−1, −0.0001]</td>
<td>0.5σ</td>
<td>&gt;0</td>
<td>−0.5σ/−0.1σ</td>
<td>NS</td>
<td>AA</td>
</tr>
<tr>
<td>KeyRegion+Protect</td>
<td>≤1%</td>
<td>(−1, 0)</td>
<td>1.0σ</td>
<td>&gt;0</td>
<td>−0.5σ/−0.1σ</td>
<td>NS</td>
<td>AA</td>
</tr>
<tr>
<td>Common+Protect</td>
<td>&gt;5%</td>
<td>(−1, 0)</td>
<td>0.1σ</td>
<td>&gt;0</td>
<td>−0.5σ/−0.1σ</td>
<td>NS</td>
<td>AA</td>
</tr>
</tbody>
</table>

*Rare variant = disease caused by multiple rare deleterious variants. Low frequency variant = disease caused by multiple low frequency deleterious variants. Key Region variant = rare deleterious variants are localized to key regions. Common variant = disease caused by a single deleterious common variant. The etiologies Rare+Protect, LowFreq+Protect, KeyRegion+Protect and Common+Protect were identical to the first four except that they include protective variants.*

1Minor allele frequency of deleterious causal variants.
2Selection coefficients of deleterious causal variants.
3Effect size of deleterious causal variants.
4Selection coefficient of protective causal variants.
5Effect size of protective modifier variants.

*−0.5σ for protective modifier variants with AF < 5%. −0.1σ for protective modifier variants with AF > 5%.

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the allele frequency spectrum is more similar to the African-
American demographic model, BOMP may be able to detect
causal variation in larger gene sets, given the size of current whole-
exome studies.

To test this hypothesis, we considered a case-control study of
200 individuals, using a set of 100 candidate genes as the
functional group. The disease etiologies of causal genes were
sampled from three categories with the ratio of 10 (Rare variant or
Low frequency variant or Key region variant) : 1 (Common
variant) : 1 (any etiology involving protective variants). Etiologies
were sampled with equal probability within each category. As
before, we varied the ratio of causal to non-causal genes in the set
from 1:3, 1:1, 3:1. BOMP power was > 0.8 for all scenarios (0.852
for 1:3, 0.956 for 1:1 and 0.956 for 3:1).

Relative contributions of mutation burden and mutation
position distribution in simulated case-control studies

We computed average power for single candidate gene case-
control studies and multiple candidate gene case-control studies
(nine genes, 3-6 causal to non-causal ratio), with respect to both
demographic models, all disease etiologies (Table 1) for single
genes, and all combinations of disease etiologies for gene sets
(Figure S3). BOMP’s hybrid likelihood model had better power
than either of its components: the mutation burden and mutation
position distribution statistics (Figure 5). The burden statistic had
more power in single-gene studies, while the position statistic had
more power in the gene set studies.

In general, mutation burden tests outperform the position
distribution statistic when causal variants are rare and are not
clustered. The position distribution test outperforms burden tests
when the number of rare variants is similar in cases and controls, but
where cases and controls differ with respect to the position
distribution of the variants. To illustrate this point, we show a case
in which burden tests would miss such a difference (Figure 6). In the
genomic region shown, cases and controls each have 9 total
variants, but an informative window segmentation yields distinct
regions in which the number of variants seen in cases and
controls is substantially different. The difference between cases and controls is
also missed by SKAT, which consider variants one at a time,
because at each position the number of variants in cases and
controls is similar. (A more detailed example is shown in Figure 7.)

Both collapsing burden and position distribution tests outper-
form SKAT when causal variants are very rare. Figure S4 shows
the power of VT, SKAT, BOMP burden, and BOMP position

Figure 2. Power estimates for multiple gene case-control studies with causal variants equally likely to be from any disease etiology
dominated by rare variants. A,B. X-axis shows number of candidate genes in 250 simulated case-control studies (approximately one-third each
from disease etiologies Rare, LowFreq and KeyRegion). All genes contain causal variants. For each method, average power is shown. Power increases
for all methods as the number of candidate genes with causal variants increases. C,D. X-axis shows the number of candidate genes and the ratio of
genes containing causal variants to those that do not contain causal variants. As the ratio decreases, the power of the tested methods also decreases.
(Tested methods are BOMP, VT, SKAT and KBAC1P = minor allele frequency defined as <1%, KBAC5P = minor allele frequency defined as <5%).
AA = the case-control studies were drawn from gene populations generated with an African-American simple bottleneck demographic model.
EA = the case-control studies were drawn from gene populations generated with a European-American exponential growth demographic model.)
doi:10.1371/journal.pgen.1003224.g002

AA simple bottleneck model

EA exponential growth model

AA simple bottleneck model

EA exponential growth model

Hybrid Likelihood Model for Disease Association

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distribution in 10,000 simulated European-American individuals, using our Rare and Key Region Variant disease etiologies. The European-American populations contain a large fraction of rare variants (Figure S2 shows exact allele frequencies and raw counts). Our simulations of the Rare Variant etiology in this population generate rare variants that are not clustered, and the methods with highest power are VT burden and BOMP burden tests. In Key Region Variant simulations where rare variants are positioned differently in cases and controls, the position distribution statistic has higher power than either of these burden tests.

Type 1 error
Because we used permutation to compute p-values, type I error should be well controlled.

Dallas Heart Study
We applied the BOMP hybrid likelihood model to the analysis of data from the Dallas Heart Study (DHS) [17]. Romeo et al. explored genetic contributions to plasma triglyceride (TG) levels in ~3500 individuals in the DHS, by resequencing the coding regions of several genes, including angiopoietin-like (ANGPTL) family genes. The ANGPTL genes regulate the activity of a key enzyme in TG metabolism, lipoprotein lipase (LPL), via post-transcriptional modifications and were jointly associated with low triglyceride levels by [17]. Specifically, ANGPTL3, ANGPTL4, and ANGPTL5 were functionally validated as causal genes, playing non-redundant roles and underlying TG levels as a functional group [17]. The ANGPTL gene set has been analyzed in several computational papers and used as a benchmark to compare methods that predict the impact of rare and common variants from sequencing data [10,12,13,20–23].

We stratified the DHS samples by ethnicity (Hispanic, non-Hispanic white, non-Hispanic black) and gender. Because BOMP was designed for dichotomous phenotypes, we selected the lower and upper quartiles from each group, by TG level (totaling 1775 individuals, with 897 cases and 878 controls).
Sixty mutations in ANGPTL3, ANGPTL4, and ANGPTL5 occurred in these individuals.

We computed a P-value for each of the three ANGPTL genes and for the ANGPTL gene set, using BOMP (with and without bioinformatics scores), the burden statistic VT (with and without bioinformatics variant weighting), the overdispersion statistic SKAT, and the mixture-model KBAC statistic (with four parameter settings) (Table 2).

The hybrid BOMP test, with bioinformatics scores and allele frequency variant weighting, had the most significant P-value for...
Figure 6. Example variation pattern in which position distribution outperforms burden tests. A toy example of a genomic region containing variants (blue squares) in cases and controls. We assume that the region is important for phenotype. Variant counts in controls (red), Variant counts in controls (purple). Cases and controls each have a total of 9 variants in this region, so Burden statistics (e.g., VT or BOMP burden) will not be able to detect that the region is important for phenotype. BOMP's position distribution statistic collapses variants into short, localized windows (red dashed lines) and detects that the number of variants seen in cases and controls is different within the windows. We note that a method that does not collapse variants, such as SKAT, does not have much power to detect the difference between cases and controls, because at each position the number of variants in cases and controls is similar.

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bias resulting from the version changes in exome capture and change from Illumina GAII to HiSeq2000 sequencing platforms between rounds one and two. We performed PCA analysis with EISENSTRAT [28], which revealed no significant differences in the nature or frequency of variants identified in the two rounds (Figure S6).

**Computational time**

In our simulations, analysis of an average-sized gene (500 codons), using 100,000 permutations, required wall-clock times of 32 s, 57.8 s, 1 m23 s, and 3 m4.2 s for case-control study sizes of 200, 1000, 2000, or 5000 (African American demographic model) and 22.8 s, 1 m7.6 s, 1 m25.2 s, and 3 m9.6 s for European American demographic model. For our gene set analyses with real data from the bipolar case-control study (298 individuals), using 100,000 permutations to compute P-values, BOMP computation time ranged from 10 m for a gene set with 101 genes to 4 h16 m for a gene set with 648 genes (Table 3). All computations were done on a machine with four dual-core 2.6 GHz AMD Processors and 16 Gb memory.

**Discussion**

In this work, we introduce and explore the power of a new hybrid likelihood model BOMP to detect causal variants.
<table>
<thead>
<tr>
<th>Gene Set Name (Source)</th>
<th>BOMP P-value</th>
<th>BOMP Burden P-value</th>
<th>BOMP Position P-value</th>
<th>FDR*</th>
<th>Synaptic Genes</th>
<th>Gene Set Size</th>
<th>Time (m)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPK SIGNALING PATHWAY (KEGG)</td>
<td>0.0065</td>
<td>1.0000</td>
<td>0.0043</td>
<td>0.0949</td>
<td>68</td>
<td>267</td>
<td>87</td>
</tr>
<tr>
<td>AXON GUIDANCE (Reactome)</td>
<td>0.0162</td>
<td>0.5847</td>
<td>0.0137</td>
<td>0.0949</td>
<td>71</td>
<td>161</td>
<td>99</td>
</tr>
<tr>
<td>NEUROLOGICAL SYSTEM PROCESS (GO)</td>
<td>0.0274</td>
<td>0.2787</td>
<td>0.0273</td>
<td>0.0949</td>
<td>75</td>
<td>377</td>
<td>177</td>
</tr>
<tr>
<td>METABOLISM OF PROTEINS (Reactome)</td>
<td>0.0299</td>
<td>0.3437</td>
<td>0.0272</td>
<td>0.0949</td>
<td>96</td>
<td>213</td>
<td>46</td>
</tr>
<tr>
<td>NEUROACTIVE LIGAND RECEPTOR INTERACTION (KEGG)</td>
<td>0.0309</td>
<td>0.8490</td>
<td>0.0259</td>
<td>0.0949</td>
<td>21</td>
<td>272</td>
<td>104</td>
</tr>
<tr>
<td>HUNTINGTONS DISEASE (KEGG)</td>
<td>0.0312</td>
<td>0.5408</td>
<td>0.0266</td>
<td>0.0949</td>
<td>77</td>
<td>185</td>
<td>51</td>
</tr>
<tr>
<td>CALCULUM SIGNALING PATHWAY (KEGG)</td>
<td>0.0332</td>
<td>0.4796</td>
<td>0.0303</td>
<td>0.0949</td>
<td>51</td>
<td>178</td>
<td>85</td>
</tr>
<tr>
<td>POST TRANSLATIONAL PROTEIN MODIFICATION (GO)</td>
<td>0.0635</td>
<td>0.4031</td>
<td>0.0620</td>
<td>0.1587</td>
<td>87</td>
<td>462</td>
<td>244</td>
</tr>
<tr>
<td>NERVOUS SYSTEM DEVELOPMENT (GO)</td>
<td>0.0744</td>
<td>0.8912</td>
<td>0.0674</td>
<td>0.1654</td>
<td>88</td>
<td>382</td>
<td>157</td>
</tr>
<tr>
<td>SIGNALING BY NGF (Reactome)</td>
<td>0.1290</td>
<td>0.8393</td>
<td>0.1139</td>
<td>0.2317</td>
<td>71</td>
<td>215</td>
<td>103</td>
</tr>
<tr>
<td>GNRH SIGNALING PATHWAY (KEGG)</td>
<td>0.1313</td>
<td>0.7485</td>
<td>0.1109</td>
<td>0.2317</td>
<td>34</td>
<td>101</td>
<td>43</td>
</tr>
<tr>
<td>OXIDATIVE PHOSPHORYLATION (KEGG)</td>
<td>0.1390</td>
<td>0.4222</td>
<td>0.1263</td>
<td>0.2317</td>
<td>66</td>
<td>133</td>
<td>21</td>
</tr>
<tr>
<td>ALZHEIMERS DISEASE (KEGG)</td>
<td>0.2164</td>
<td>0.7012</td>
<td>0.1904</td>
<td>0.3151</td>
<td>76</td>
<td>169</td>
<td>46</td>
</tr>
<tr>
<td>INTRACELLULAR SIGNALING CASCADE (GO)</td>
<td>0.2267</td>
<td>0.7489</td>
<td>0.2174</td>
<td>0.3151</td>
<td>120</td>
<td>648</td>
<td>256</td>
</tr>
<tr>
<td>NEUROTROPHIN SIGNALING PATHWAY (KEGG)</td>
<td>0.2363</td>
<td>0.7995</td>
<td>0.2031</td>
<td>0.3151</td>
<td>45</td>
<td>126</td>
<td>44</td>
</tr>
<tr>
<td>CHEMOKINE SIGNALING PATHWAY (KEGG)</td>
<td>0.2667</td>
<td>0.6638</td>
<td>0.2464</td>
<td>0.3272</td>
<td>50</td>
<td>190</td>
<td>62</td>
</tr>
<tr>
<td>WNT SIGNALING PATHWAY (KEGG)</td>
<td>0.2781</td>
<td>0.7009</td>
<td>0.2519</td>
<td>0.3272</td>
<td>38</td>
<td>151</td>
<td>56</td>
</tr>
<tr>
<td>REGULATION OF GENE EXPRESSION IN BETA CELLS (Reactome)</td>
<td>0.3401</td>
<td>0.0877</td>
<td>0.4571</td>
<td>0.3779</td>
<td>60</td>
<td>101</td>
<td>10</td>
</tr>
<tr>
<td>MITOCHONDRIAL (GO)</td>
<td>0.4315</td>
<td>0.9727</td>
<td>0.4047</td>
<td>0.4542</td>
<td>117</td>
<td>339</td>
<td>104</td>
</tr>
<tr>
<td>PARKINSONS DISEASE (KEGG)</td>
<td>0.7893</td>
<td>0.3087</td>
<td>0.8111</td>
<td>0.7893</td>
<td>62</td>
<td>133</td>
<td>22</td>
</tr>
</tbody>
</table>

The gene sets were selected for testing because they contained > 100 genes and were the most significantly enriched by synaptic genes [26]. Seven of the gene sets were nominally associated with bipolar disorder (P < 0.05) and have FDR < 0.1. *FDR computed with the Benjamini-Hochberg algorithm [45]. **Wall-clock time in minutes.
underlying dichotomous disease phenotypes. We compared its power with that of several leading methods designed to detect causal variation in whole-exome case-control studies. We performed simulated case-control studies, using a variety of sizes, demographic models, and disease etiologies. The hybrid BOMP model had good power compared to several popular methods (Figure 1, Figure 2, Figure 3). Its strengths were most apparent when we tested gene sets for association with phenotype, when protective or common variants were present, and when a phenotype was associated with variants that cluster in key regions on a gene.

Because no current variant collapsing methods have been shown to be best for every disease etiology [6], we were particularly interested in how the performance of BOMP and other methods varied by etiology. Our case-control study simulations were designed to represent eight etiologies (Table 1). Etiologies were defined by properties of their causal variants, based on range of minor allele frequencies, selection coefficients, position distribution, and mean effect sizes. Some etiologies include protective variants, while others include only deleterious variants. For all of our etiologies, only coding, non-silent variants were considered to have an impact. Importantly, our etiologies are defined in a probabilistic way since we allow for a randomly selected fraction of variants which meet the causality criteria for an etiology to have no effect (Methods). As a result, in the rare variant etiology, all causal variants are rare, but not all rare variants are causal, etc.

Method performance also varied by the population demographic models (European-American exponential growth and African-American simple bottleneck) used in our simulations. We provide details of how the performance of all tested methods was affected by disease etiology and demographic model in Text S1.

**Gene set simulations**

We considered the possibility that differences between cases and controls might be detected with respect to a gene set, rather than a single gene [29]. We tested the methods, using gene sets of different sizes (Figure 2), different combinations of disease etiologies (Figure 3, Figure S3), and different fractions of genes containing causal variants (Figure 2). Of the tested methods, burden statistics and KBAC were the least effective at detecting gene sets containing causal variants. SKAT and the BOMP position distribution statistic were the most effective.

Biologically, we don’t expect that every gene in a real gene set will contain causal variants. Thus our simulated gene sets were designed to contain a mix of genes with causal variants and those without. The burden tests (VT and BOMP burden) were not able to effectively capture the difference between the two and lost power as the number of non-causal variants in the simulations increased (Figure 2). The genotype vectors computed by KBAC become larger and more heterogeneous when applied to a gene set, rather than a single gene. Thus, the KBAC strategy of leveraging the number of shared genotype vectors among cases and/or controls is less effective when applied to gene sets than to single genes. The BOMP hybrid likelihood statistic (with strong contributions from the BOMP position distribution statistic), and SKAT were the most powerful when applied to gene sets, rather than single genes. We attribute this result to the increase in the number of significant localized units in a gene set that contains more than one causal gene.

We found in our simulations that a case-control study size of 1000 individuals (500 cases, 500 controls) BOMP was sufficiently powered to detect causal variants in situations when a good candidate gene set (of approximately 25 genes) was known. However, if the ratio of causal to non-causal genes in the selected gene set was low (1:3) and/or the individuals in the study carried a high proportion of rare (MAF <1%) or singleton variants, the power of the BOMP hybrid model, like all the methods examined, was diminished. This result highlights the importance of very high quality gene sets derived either from disease experts or improved bioinformatics tools for success in real data analysis.

**Dallas Heart Study benchmark set**

When we applied BOMP, VT, SKAT, and KBAC to an empirical dataset, each method displayed both strengths and weaknesses. While the dataset is small, it is interesting to note that P-values of association between variant ANGPTL family genes and dichotomized serum triglyceride levels from the Dallas Heart Study were most significant for the BOMP hybrid model, when the genes were considered together as a gene set. However, the burden statistic VT had the most significant P-value for ANGPTL3, and the KBAC P-value was the most significant for ANGPTL3 (specifically with single directional scoring and threshold for rare variation set at MAF <1%). For ANGPTL4, the most significant P-values were from the BOMP position distribution score, VT, and SKAT. Each of these genes had a different pattern of variation frequencies in cases and controls, which presented advantages and obstacles for each method. For example, ANGPTL3 had a high frequency variant (M259T) that occurred more often in cases than controls and many “noisy” rare/singleton variants that occurred either in cases or controls. VT took advantage of the signal in M259T because its threshold adapted to maximize the burden increase in cases versus controls, and thus M259T was included in its burden calculation. BOMP’s burden statistic did not give as much importance to M259T, because it downweights high frequency variants. KBAC included M259T only when its allele frequency threshold parameter was set to 5% but was penalized when it was set to 1%.

BOMP is not designed to be adjusted for additional covariates, which are often available in disease studies. For example, it is not designed to explicitly deal with different ancestries in a structured population. However, if the true population structure is known and the number of subpopulations is not too large, we can run analyses with stratification to get around this problem, as we (and the authors of the VT and SKAT papers) did for ANGPTL family genes in the Dallas Heart Study [10,12]. Using this strategy, one begins with a quantitative trait (serum triglyceride levels), stratifies individuals into groups, then identifies extreme phenotype individuals from each group. Cases are then those individuals from all groups at one extreme and controls are those individuals from all groups at the other extreme. An alternative strategy is to permute case-control labels only within each group to generate a correct null distribution.

Incorporating bioinformatics scoring of variants (by VEST) yielded improved P-values for both BOMP and VT on the Dallas Heart Study data. While it has been suggested that bioinformatics misclassification of variants might be more of a liability than a benefit, our results (albeit on a small gene set) suggest the opposite. Functional classification of variants in both coding and non-coding regions of the genome is an active research area in bioinformatics, and as methods improve, it is likely that they will increasingly contribute to statistical analysis of causal variation.

**Bipolar whole-exome sequencing, case-control study**

Finally, we applied BOMP (with VEST scoring) to test candidate gene sets in data from an on-going whole-exome study of bipolar disorder. The top gene set was the “MAPK signaling pathway” defined from the KEGG Pathway Database.
Arachidonic acids are involved in brain signaling processes that regulate the intensity and duration of MAPK activity and by helping to shuttle MAPK between the cytoplasm and nucleus, has been associated with bipolar disorder [32,33]. There are several other genes of considerable interest in this gene set, as they have been implicated previously in the genetics of bipolar disorder. For example, this pathway also containsCACNA1C and other members of the family of voltage gated calcium ion channels. CACNA1C was recently implicated as one of the most significant genes in a mega-analysis of genome-wide association studies (GWAS) of bipolar disorder carried out by the Psychiatric GWAS Consortium (PGC) [34]. This gene set also contains the phospholipase A2 family of genes. Phospholipases mediate the release of arachidonic acid from membrane phospholipids. Arachidonic acids are involved in brain signaling processes that mediate a number of effects, and there is growing evidence that the arachidonic acid signaling cascade may be a common target of multiple diverse mood stabilizers [35].

Complex diseases are expected to have considerable genetic heterogeneity i.e., they may be the consequence of alterations in hundreds of potentially causal genes, and affected individuals may have causal and/or protective variants in different subsets of these genes. The simulations done in this work reflect this heterogeneity (example in Figure S7), and we still retain good power.

Bayesian extensions of our work could include prior knowledge about the probability that a functional group of interest is associated with the phenotype. For example, if the functional group is a gene, prior evidence could come from expert knowledge based on previous functional and/or case-control studies. By combining the log likelihood ratio (Equation 1) with a log prior probability, we could estimate the log ratio of the full posterior distributions.

In summary, we have developed a new method for identifying causal variants in high-throughput sequencing data from case-control studies. It is shown to have good power relative to other leading methods and can be flexibly used in a variety of realistic scenarios. The genetic architecture of most common human diseases is likely complex, involving variants with a wide spectrum of frequencies from rare to common and contributing to disease through a number of inter-related pathways. The emergence of whole-exome and genome sequencing studies promises to accelerate our ability to interrogate the genetic architecture of these disease. However, a major challenge remains how to make sense of the enormous amounts of data generated by such studies. Our new method provides another useful tool in a growing toolbox for analyzing the data from such studies.

### Methods

**BOMP (Burden Or Mutation Position statistics),** the hybrid likelihood model proposed here, consists of two likelihood ratio tests (mutation burden and mutation position distribution statistics) with the same general form,

\[
L_j = \log \left( \frac{L(FG_j | H_A)}{L(FG_j | H_0)} \right)
\]

and tests the evidence for the alternative hypothesis \( H_A \) that a functional group (FG) of interest is associated with a disease phenotype, compared to the null hypothesis \( H_0 \) that they are not associated. Higher values of \( L_j \) indicate stronger association between unit \( j \) and the disease phenotype. In this work, the functional groups of interest are either single genes or sets of multiple genes.

### BOMP mutation burden statistic

The first likelihood ratio test is based on comparing mutation burden in cases and controls. Each individual is represented with a Bernoulli random variable, which is 1 if the individual’s burden exceeds a burden threshold, and 0 otherwise. To model the likelihood, we assume that individual burden status is independent and identically distributed (IID). The ratio compares an alternative hypothesis that the probability of exceeding the burden threshold is higher in cases than in controls and the null hypothesis (that probabilities are equal or lower in cases than in controls). Biologically, the IID assumption is not necessarily true. We control for such violations by assessing the statistical significance of the likelihood ratio by permuting case and control labels (Figure S8).

**Individual burden.** For individual \( k \) the gene burden of \( g_j \) is

\[
S_{g_j,k} = \sum_{i=1}^{n_{j,k}} x_{i,k},
\]

where \( n_{j,k} \) is the number of variants carried by individual \( k \) in gene \( j \) and \( x_{i,k} \) is the genotype of variant \( v_i \).

**Individual burden thresholds.** A binary variable is used to label individuals whose mutation burden in a gene of interest exceeds a critical threshold. If the burden of gene \( g_j \) in individual \( k \) is greater than or equal to the threshold \( t \), then it is considered to be disease phenotype associated for that individual and \( Y_{g_j,k} = 1 \) (0 otherwise). Because genes are heterogeneous in size, functional importance, mutation rate, and tolerance to variation, each gene may have a different value of \( t \). For each gene \( j \), this cutoff \( t_j \) is computed by iterating over all cut-offs and selecting the one that maximizes its mutation burden statistic (Equation 4).

**Aggregated burdens.** The \( Y_{g_j,k} \) values are then aggregated by summing over cases and controls:

\[
T_j^C = \sum_{k \in \text{cases}} Y_{g_j,k}
\]

\[
T_j^U = \sum_{k \in \text{controls}} Y_{g_j,k}
\]

The maximum likelihood estimate of the probability that the mutation burden of gene \( j \) exceeds the threshold in cases is then

\[
\hat{p}_j^C = \frac{1}{m+2} \left( T_j^C + 1 \right),
\]

and the estimate for controls is

\[
\hat{p}_j^U = \frac{1}{l+2} \left( T_j^U + 1 \right)
\]

where \( m \) is the number of cases and \( l \) the number of controls. The probability estimates \( \hat{p}_j^C, \hat{p}_j^U \),
and \( \hat{p}_j \) are used as the parameters of three Bernoulli distributions (one for cases, one for controls, and one for cases and controls together). Pseudocounts are added to avoid zero counts. The aggregated burden calculation (without pseudocounts) is illustrated in Figure 8 (and Figure 7).

**Burden likelihood ratio statistic.** For a gene \( g_j \) the mutation burden statistic is defined as a ratio of Bernoulli likelihoods:

\[
\Lambda_B(g_j) = \log \left( \frac{L_B^A \times L_B^U}{L_B^{A+U}} \right)
\]

\[
= \log \left( \frac{\left( \hat{p}_j \right)^{T_j^A} \left( 1 - \hat{p}_j \right)^{m - T_j^A} \left( \hat{p}_U \right)^{T_j^U} \left( 1 - \hat{p}_U \right)^{l - T_j^U}}{\left( \hat{p}_j \right)^{T_j} \left( 1 - \hat{p}_j \right)^{m + l - T_j^A - T_j^U}} \right)
\]

where \( m \) is the number of cases, \( l \) the number of controls; \( T_j^A \) is the number of cases whose mutation burden in \( g_j \) exceeds an optimized threshold (Individual Burden Thresholds); \( T_j^U \) is the number of controls exceeding the threshold; \( \hat{p}_j \) is the maximum likelihood estimate of the probability that the burden of gene \( j \) exceeds the threshold in cases, \( \hat{p}_U \) is the estimate that gene \( j \) exceeds the threshold in controls, \( \hat{p}_j \) is the estimate that gene \( j \) exceeds the threshold in both cases and controls. First, we consider only genes with higher burden in the cases, for which \( \hat{p}_j \geq \hat{p}_U \).

Next, for the remaining genes, we modify Equation 4,

\[
\Lambda_B(g_j) = \log \left( \frac{\left( \hat{p}_j \right)^{T_j^A} \left( 1 - \hat{p}_j \right)^{m - T_j^A} \left( \hat{p}_U \right)^{T_j^U} \left( 1 - \hat{p}_U \right)^{l - T_j^U}}{\left( \hat{p}_j \right)^{T_j} \left( 1 - \hat{p}_j \right)^{m + l - T_j^A - T_j^U}} \right)
\]

Thus genes with higher burdens in cases than controls get a high value and those with higher burdens in controls than cases get a low value.

It follows that under \( H_A \), the number of cases in which the burden of gene \( j \) exceeds the threshold will be larger than in controls, and that under \( H_B \), they will not be different.

If a gene set rather than a single gene is used as the functional group, the burden is aggregated across all genes in the set, and the procedure is otherwise identical.

**BOMP mutation position distribution statistic**

The second likelihood ratio test is based on comparing the position distribution of mutations in cases and controls. The codons of a gene are partitioned into windows and mutation count (burden score) is computed for each window in cases only, controls only, and in cases and controls together. To model the likelihood, each window mutation count is considered to be a random variable in a multinomial distribution. If the partition contains \( d \) windows, there are \( d \) possible outcomes for each mutation. There are also \( d \) multinomial parameters for the partition.

**Window mutation counts.** Let the window mutation counts in the multinomial distributions be \( W_{x_j}^A \), \( W_{x_j}^U \), and \( W_{x_j}^{A+U} \) (cases only, controls only, and in cases and controls together) for cases \( W_{x_j}^A = \sum_{k \text{cases}} S_{x_j,k} \), for controls \( W_{x_j}^U = \sum_{k \text{controls}} S_{x_j,k} \), for cases and controls \( W_{x_j}^{A+U} = \sum_{k \text{cases and controls}} S_{x_j,k} \).


where \( S_{x_j,k} \) (computed as in Equation 2) is the score for individual \( k \) in window \( x \).

The maximum likelihood estimate of the multinomial parameters (including pseudocounts) is then

\[
\hat{p}_x^A = \frac{W_{x_j}^{A+1}}{\sum_x (W_{x_j}^A + 1)}
\]

\[
\hat{p}_x^U = \frac{W_{x_j}^{U+1}}{\sum_x (W_{x_j}^U + 1)}
\]

\[
\hat{p}_x^{A+U} = \frac{W_{x_j}^{A+U+2}}{\sum_x (W_{x_j}^{A+U} + 2)}
\]

**Position distribution likelihood ratio statistic.** For a gene \( g_j \), the statistic is defined as a ratio of multinomial likelihoods:

\[
\Lambda_P(g_j) = \log \left( \frac{L_P^A \times L_P^U}{L_P^{A+U}} \right)
\]

where

for cases \( L_P^A = \prod_x \left( \hat{p}_x^A \right)^{W_{x_j}^{A+1}} \)

for controls \( L_P^U = \prod_x \left( \hat{p}_x^U \right)^{W_{x_j}^{U+1}} \)

for cases and controls \( L_P^{A+U} = \prod_x \left( \hat{p}_x^{A+U} \right)^{W_{x_j}^{A+U+2}} \).


It follows that under \( H_A \), the likelihood for cases will be different than for controls, and that under \( H_B \), they are not different. In contrast to the mutation burden statistic, there is no directionality in the position mutation distribution statistic, because \( \Lambda_P(g_j) \) will be large when either \( \hat{p}_x^A \) or \( \hat{p}_x^U \) is large.

A toy example of aggregated window mutation count calculation is illustrated in Figure 8 (and Figure 7).

**Windows and sequence segmentations.** Each gene has many possible window partitions, and we don’t know in advance which is the most informative for the position distribution statistic. One way to create candidate window partitions (i.e., sequence segmentations) for a gene of length \( L \) is to select a window size \( s \) and a series of possible offsets, based on a selected shift increment \( t \). Each offset generates a new segmentation (Figure S9). For
Figure 8. Components of BOMP Hybrid Likelihood Model compared. A. Mutation burden statistic. The Mutation burden statistic uses the aggregated burden for cases, $T_A^j$, and controls $T_U^j$. B. Mutation position distribution statistic. Aggregated window mutation counts are calculated for cases, $W^{A+U}_j$; controls, $W^U_j$; and cases and controls combined, $W^{A+U}_j$, across $x$ windows.

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example, if the window size is 8 and the shift increment is 1, the first offset begins at the first position of the gene and generates a segmentation of \( \left\lfloor \frac{L}{8} \right\rfloor \) windows. The second offset will begin at position 2 of the gene and generate a new segmentation of \( \left\lfloor \frac{L-1}{8} \right\rfloor + 1 \) windows, etc. In this work we used four combinations of window size \( s \) and shift increment \( t \): \((8,1), (16,2), (32,4)\) and \((64,8)\), yielding 32 candidate segmentations for a gene. These choices were not optimized and can be adjusted, according to user preference and/or prior knowledge. The best segmentation is selected by computing the likelihood ratio \( \Lambda(g_j) \) (Equation 9) for each segmentation and picking the segmentation with the largest \( \Lambda(g_j) \). Alternatively, this likelihood ratio can be modified by computing \( W_{x,r}^A \), \( W_{x,r}^U \) and \( W_{x,r}^{A+U} \) with respect to total positions mutated, rather than total number of mutations.

For the position distribution statistic, if a gene set rather than a single gene is used as a functional group, the best window segmentation is computed for each gene, and the calculation of the position distribution statistic is otherwise identical.

The mutation burden and mutation position burden statistics are combined into a single log likelihood ratio,

\[
\Lambda_{bj} = \Lambda_B(g_j) + \Lambda_P(g_j). \tag{13}
\]

Controlling false positives through permutation

P-values for each \( \Lambda_{bj} \) are computed with a null distribution, generated by repeated permutation of case and control labels. All parameters of \( \Lambda_B(g_j) \) and \( \Lambda_P(g_j) \), including the maximum likelihood burden threshold and segmentation pattern, are calculated initially for empirical data and then re-calculated for each iteration of the permutation. Thus, \( N \) iterations yields \( N \) null \( \Lambda_{bj}^{(n)} \) where \( n \) ranges from 1 to \( N \) (Figure S8, Figure S10).

The permutation controls for confounding effects, such as properties that characterize a particular gene or region of interest (i.e., nucleotide diversity, GC content, and recombination rate), which are the same when used to estimate \( \Lambda_{bj} \) and each null \( \Lambda_{bj}^{(n)} \).

After \( N \) iterations (e.g., \( N = 10^6 \))

\[
P_{bj} = \frac{\# \left( \text{null } \Lambda_{bj}^{(n)} \geq \Lambda_{bj} \right) + 1}{N + 1} \tag{14}
\]

While \( \Lambda_B(g_j) \) and \( \Lambda_P(g_j) \) are not independent, using the permutation test yields an accurate P-value estimate, because any dependencies in \( \Lambda_{bj} \) are reproduced in each null\( \Lambda_{bj}^{(n)} \).

Extensions to the basic method

Genetic models. Either dominant or additive genetic models can be specified. Under the dominant genetic model, both homozygous and heterozygous variants have \( x_i = 1 \); under the additive model, homozygous variants have \( x_i = 2 \) and heterozygous variants \( x_i = 1 \). For all experiments in this work, additive models were used.

Variant scores. The Individual Burden can be modified by incorporation of score coefficients so that \( S_{g_j} = \sum_{i=1}^{N_{g_j}} x_i \cdot k_i \). The score for a variant \( v_i \) can be either a bioinformatics-based score \( s_i = f_i \), an allele-frequency-based score \( s_i = \frac{1}{\sqrt{\theta_i(1-\theta_i)}} \), following [9,10]; or the product of both \( s_i = f_i \frac{1}{\sqrt{\theta_i(1-\theta_i)}} \). Next we explain how these scores are calculated. In this work, variant scores were used only in the burden statistic. The allele-frequency-based score was used for all simulations and the product of allele-frequency and bioinformatics scores was used on all empirical data.

Bioinformatics scores. Each nonsilent variant \( v_i \) can be assigned a score \( f_i \in [0,1] \) to represent its contribution to a disease phenotype of interest, where \( f_i = 0 \) indicates no contribution and \( f_i = 1 \) indicates a strong contribution. These scores are estimated with Variant Effect Scoring Tool (VEST). Variants causing nonsense, nonstop or frameshift alterations to a gene’s protein product receive \( f_i = 1 \). Variants causing missense alterations are scored with a Random Forest classifier [36,37]; the score is the fraction of decision trees in the forest that classified the variant as deleterious. Alternatively, other bioinformatics methods that score missense variants can be used to generate \( f_i \) values, if scaled to range from 0 to 1.

The Variant Effect Scoring Tool is a Random Forest classifier, trained with the CHASM software suite’s Classifier Pack and SNVBox [30]. The Forest contains 1000 decision trees. The positive class of 45,000+ missense variants is taken from the Human Gene Mutation Database (HGMD) [39]. The negative class of 45,000+ missense variants is randomly selected from variants validated by the 1000genomes project [40] in the SNP135 table of the UCSC Genome Browser database [41]. Each missense variant is represented by 86 features in SNVBox, including conservation scores, amino acid residue substitution scores, UniProtKB annotations [42], and predicted local protein structure.

Allele-frequency-based scores. For each \( f_i \), we estimate its mean population allele frequency \( \theta_i \) as follows:

\[
E[\theta_i] = \frac{x_i^A + x_i^U + 1}{2N + 2} \tag{15}
\]

where \( x_i^A \) and \( x_i^U \) are allele counts of variant \( v_i \) in cases and controls, respectively; \( N \) is the number of individuals in both cases and controls; and the constants are pseudocounts from a beta prior.

Simulation framework

Simulated case-control studies are generated using two demographic growth models, eight disease etiologies, and a stochastic model of genotype-phenotype association.

Generating genomic populations. The general Wright-Fisher model/forward population genetic simulation tool SFS_code [43] is used to generate 100 effective genomic populations and to sample 1,000,000 haplotypes per population. As in [13], the simulated haplotypes in each population are randomly paired to generate 500,000 diploid individuals. Two demographic growth structures are used: an exponential growth model fitted to deep resequencing data from European Americans [19] and a simple bottleneck model fitted to whole-genome polymorphism data from African-Americans [44]. For the exponential growth demographic model, distribution of fitness effects of new mutations (DFE) is modeled with a two-component gamma distribution (similar to [19]). For the simple bottleneck model, DFE was modeled as described in [44] (Text S1:Demographic Models). Following [19], the mutation rate is set to \( 1.8 \times 10^{-8} \) per generation for all simulations.

Generating phenotypic traits for individuals with a single causal gene. The individuals in a population are then
associated with a quantitative phenotypic trait, which we assume drives a disease, so that individuals with high values of the trait will have the disease and those with low values of the trait will not. Eight possible disease etiologies are considered (Table 1). Each etiology is defined by properties of its causal variants. Variants can be rare, low frequency, or common. They can occur in any gene or region. They can occur in any key region or variant. These variants can be functional or non-functional. They can be causal or non-causal. They can be present or absent. In this work, only coding, non-synonymous variants are considered as causal or protective for all etiologies. (However, disease etiologies that consider silent variants, which impact gene regulation, could also be defined.) For etiologies with key region variants, we used haplotypes that contained multiple coding segments (100 segments, each 30 bases long). Otherwise haplotypes contained a single coding segment of 1500 bases.

To generate the phenotypic traits for the genomic populations, we select a disease etiology and a population that contains variants meeting the criteria for causality in that etiology (Table 1). Next the quantitative disease trait $QT$ is generated for each individual in the population, using an approach based on [19]. Trait values are drawn from Gaussian distributions, such that individuals with no causal variants have

$$QT \sim N(\mu, \sigma), (\mu, \sigma) = (0, 1).$$

(16)

Individuals with $n$ causal variants have

$$QT \sim N(\mu + nk\sigma, \sigma), (\mu, \sigma) = (0, 1).$$

(17)

where $k$ is the mean shift in trait value (the shift per variant) for an individual.

To match the expected effect size of significant common and rare variants in GWAS, effect sizes of $0.1\sigma$ for common variants (Text S1: Effect size of common variants) and $0.5\sigma - 1.0\sigma$ for rare variants are used. The strongest effects are $1.0\sigma$ for rare variants in Key Regions. We assume that these variants occur at functionally important positions and that in a gene of interest, they are unlikely to occur more than once in a single individual. Our choice of effect size is somewhat larger than that used by Kryukov et al. [19], who use $0.25\sigma - 0.5\sigma$ for rare variants. To account for heterogeneity within a particular disease etiology, effect size can be 0 for a designated fraction of causal variants.

**Case-control study generation.** At this stage, each population consists of genomic individuals, each with a real-valued quantitative trait. To construct the case-control studies, an extreme phenotype model is used. Disease prevalence is set at 1%, i.e., the 1% of individuals in the selected population with the highest values of $QT$ are considered affected and the 25% of individuals with the lowest values of $QT$ unaffected.

Case-control studies are generated by sampling without replacement from affected and unaffected groups in a population. Individuals with intermediate phenotype values are not included in case-control studies. The random process used to generate $QT$ (Generating phenotypic traits for individuals with a single causal gene) ensures varied penetrance and phenocopy rates in each case-control study, e.g., some individuals carrying deleterious variants are not affected, while some with no deleterious variants are affected.

**Null case-control study.** A null case-control sample is also generated, with no disease etiology, in which the phenotypic trait is drawn from a standard normal distribution for every individual in the sample.

**Generalization to multiple genes.** For a scenario in which the functional group of interest is a gene set e.g., involved in a pathway or biological process, we construct a new population of 500,000 individuals, in which each individual has multiple genes. This population is created by sampling genes from the diploid gene populations generated previously (Generating genomic populations). Next, we specify a gene set size and fraction of genes in the gene set that contain causal variants. A disease etiology is then randomly selected for each gene that contains causal variants. Finally, the phenotypic trait for each individual in the population is generated using Equations 16 and 17. Gene set case-control studies were generated with the same protocol as for single causal genes.

**Supporting Information**

**Figure S1** Power estimates for BOMP, VT, SKAT, KBAC for case-control study with 10,000 individuals. (KBAC1P = minor allele frequency defined as <1%, KBAC5P = minor allele frequency defined as <5%). Each column represents power estimates for each method, based on 250 simulated case-control studies. All case-control studies had 10,000 genomic individuals, each with a single gene. AA = the case-control studies were drawn from gene populations generated with an African-American simple bottleneck demographic model. EA = the case-control studies were drawn from gene populations generated with a European-American exponential growth demographic model. The eight variant causality (disease etiology) models are defined in Table 1. Since the European-American demographic model does not account for common or protective variants, etiologies involving common or protective variants were only considered for the African-American demographic model.

(PDF)

**Figure S2** Distributions of allele frequencies and raw allele counts in simulated European-American and African-American populations. The European-American population consists almost entirely of rare variants, while the African-American population contains a wider range of rare, low-frequency, and common variants. Percentage of variants with allele frequencies and raw allele counts in the designated ranges are shown. Because >99% of European-American allele frequencies are <0.1%, we include a blow-up of frequencies >0.1%, which range from 0.05% to 0.2%. Demographic models shown in Figure S11.

(PDF)

**Figure S3** Nine multinomial distributions used to construct sets of multiple candidate genes for case-control studies. Each multinomial distribution is named for its dominant disease etiology.

(PDF)

**Figure S4** Power of position distribution statistics compared to burden methods and SKAT. Burden tests outperform the position distribution statistic when causal variants are rare and are not clustered, as in our simulations of Rare Variant disease etiology and European-American demographic. The position distribution test outperforms burden tests when the number of rare variants is similar in cases and controls, but where cases and controls differ with respect to the position distribution of the variants, as in simulations of Key Region Variant disease etiology and European-American demographic. Both collapsing burden and position distribution tests outperform SKAT when causal variants are very rare. RareEA = rare variant disease etiology (Table 1) and European-American demographic model. KeyRegionEA = key region variant disease etiology (Table 1) and European-
American demographic model. Power shown on Y-axis. Simulations with 10,000 samples are shown.

**Figure S5** Q-Q plot of BOMP P-values for all genes in the Bipolar case-control study. The plot shows no evidence of skew or heavy tails, indicating that there is no systematic bias in our analysis. Empirical P-values are below the line because the BOMP statistic is not continuous for genes with few variants in only a few samples, leading to conservative P-values.

**Figure S6** PCA plot showing the overlap of bipolar samples and controls sequenced during rounds 1 and 2. Plot obtained using EIGENSTRAT [28], where the first and second components of the model (PC1 and PC2, respectively) are shown. The analysis does not show significant differences in the nature or frequency of variants identified in the two rounds. Round 1 = Nimblegen v1.0 and Illumina GAII, Round 2 = Nimblegen v2.0 and Illumina HiSeq2000.

**Figure S7** Example of how our simulations capture genetic heterogeneity in complex disease. Each horizontal grid line represents a genomic individual. (Cases and controls shown separately.) Each vertical gridline represents a gene. Causal variants (both deleterious and protective) are shown as triangles. Different case individuals have different patterns of causal variants and the allele frequencies of the variants range from rare (1 allele) to common (190 alleles). Causal variants are also observed in the control individuals. Type = Downward pointing triangles are deleterious variants, upward pointing triangles are protective variants. (200 genomic individuals from African-American demographic model are shown).

**Figure S8** Flow chart for calculation of mutation burden statistic. The statistic is first calculated from empirical data (by following the blue and black arrows). The null mutation burden statistics are calculated by following the red and black arrows. Key steps in the calculation are: computing the burden for each individual; sorting the individual burdens into a ranked list, where \( m \) denotes list rank; selecting a candidate burden threshold; computing maximum likelihood estimates of Bernoulli parameters at this threshold; computing the LLR (log-likelihood ratio) with these parameters. Candidate burden thresholds are iteratively tested and the threshold yielding the largest LLR is returned. To generate the null distribution, case-control labels are repeatedly permuted and the key steps are followed to compute one point in the null. The permuted null distribution is estimated by repeating steps 1–4 10,000 times (or user-adjusted value) and used to compute the p-value for the statistic.

**Figure S9** Window and sequence segmentations. The mutation position distribution statistic requires a segmentation for a sequence of interest (e.g., a gene). We generate candidate segmentations by selecting a window size \( s \) and allowing a series of possible offsets, based on a selected shift increment \( t \). In this example, we illustrate the eight possible window segmentations of a gene with 24 codons (represented by rectangles), using a window size of 8 and a shift increment of 1.

**Figure S10** Flow chart for calculation of position distribution statistic. The statistic is first calculated from empirical data (by following the blue and black arrows). The null position distribution statistics are calculated by following the red and black arrows. Key steps in the calculation are: choice of (user-selected) window sizes \( s \) and shift increment \( t \) to generate a set of gene segmentations (Each unique segmentation is defined by \( s, t, \) and shift, which represents the current segmentation offset); estimation of \( d \) parameters for each of three multinomial distributions – cases, controls, and cases and controls together – (for a segmentation with \( d \) windows); computation of log likelihood ratio; finding the largest log likelihood ratio for all segmentations. To generate the null distribution, case-control labels are repeatedly permuted and the key steps are followed to compute one point in the null. The permuted null distribution is estimated by repeating steps 1–4 10,000 times (or user-adjusted value) and used to compute the p-value for the statistic.

**Figure S11** Demographic models of European-American and African-American populations. The models were fit to European-American [19] and African-American sequencing data [44].

**Table S1** BOMP, VT, and SKAT comparison. Approaches to variant collapsing, variant importance, and choice of statistical framework define differences and similarities among BOMP, VT, and SKAT. A. Variant collapsing strategies. VT and BOMP burden both collapse variants across a genomic region. SKAT does not do collapsing and considers variants one at a time. BOMP position distribution collapses variants across local windows over a genomic region. These different collapsing strategies are illustrated in a toy example in Figure 8 of our main manuscript. B. Variant importance. All methods assume that some variants are more important than others. This idea is implemented by either filtering and/or weighting variants. C. Statistical framework. VT and BOMP burden both use a summary statistic to compare burden in cases and controls and assess the statistical significance of the statistic with permutation. For VT, individual burdens are summed over the case group and over the control group and summarized by the difference in Z-score between the two groups. For BOMP burden, individual burden is dichotomized for each sample, by selecting a burden threshold. The probability of exceeding the burden threshold for cases and for controls is estimated. The difference between the two is compared with a log-likelihood ratio. For SKAT, phenotypic labels (case or control) are directly regressed for each variant. The coefficient for each variant is tested by comparing it to 0, with a variance-component score test. Statistical significance is calculated analytically. For BOMP position distribution, the difference of distributions of variants over local windows between cases and controls is modeled by multinomial likelihood and then summarized by a log-likelihood ratio.

**Text S1** Supplementary Material.

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**Author Contributions**

Conceived and designed the experiments: Y-CC RK. Performed the experiments: Y-CC HC MP. Analyzed the data: Y-CC RK HC MP PPZ. Contributed reagents/materials/analysis tools: Y-CC RK HC JP MK FSG MP PPZ WRM JBP. Wrote the paper: RK Y-CC MP PPZ HC.