Integrated analysis of variants and pathways in genome-wide association studies using polygenic models of disease

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Abstract

Many common diseases are highly polygenic, modulated by a large number genetic factors with small effects on susceptibility to disease. These small effects are difficult to map reliably in genetic association studies. To address this problem, researchers have developed methods that aggregate information over sets of related genes, such as biological pathways, to identify gene sets that are enriched for genetic variants associated with disease. However, these methods fail to answer a key question: which genes and genetic variants are associated with disease risk? We develop a method based on sparse multiple regression that simultaneously identifies enriched pathways, and prioritizes the variants within these pathways, to locate additional variants associated with disease susceptibility. A central feature of our approach is an estimate of the strength of enrichment, which yields a coherent way to prioritize variants in enriched pathways. We illustrate the benefits of our approach in a genome-wide association study of Crohn’s disease with ~440,000 genetic variants genotyped for ~4700 study subjects. We obtain strong support for enrichment of IL-12, IL-23 and other cytokine signaling pathways. Furthermore, prioritizing variants in these enriched pathways yields support for additional disease-association variants, all of which have been independently reported in other case-control studies for Crohn’s disease.

Introduction

By surveying genetic variation throughout the genome, and systematically searching for variants correlated with disease phenotypes, genome-wide association studies (GWAS) have led to the discovery of genes and genetic loci that were not previously suspected of playing a role in complex diseases [1–4]. For example, the discovery of disease-correlated variants in GWAS of Crohn’s disease, a common form of inflammatory bowel disease, has helped draw links to genes that regulate autophagy and innate immune responses [5–10].

In most analyses of GWAS, genetic associations are identified by testing each marker one at a time for association with disease. Additional clues about genetic variants, such as whether they are coding, exonic, or lie near the transcription start site of a gene, are usually considered post hoc, rather than incorporated into the statistical analysis. While such “hypothesis-free” analyses have successfully identified variants associated with disease, they have important shortcomings. Many commonly occurring diseases are believed to be modulated by a large number of genetic factors, each of which have a small effect on disease risk, so they may be difficult to identify in a GWAS [11,12]. This problem is compounded, according to some predictions [11,12,13], by the low prevalence of many alleles conferring risk to complex diseases. Motivated by these shortcomings, researchers have developed “pathway analysis” approaches to GWAS [17,21]. These methods are grounded on the theory that complex disease arises from the accumulation of genetic effects acting within common biological pathways [22,23]. A main goal of pathway analysis is to identify pathways that are enriched for disease—that is, groups of related genes that are more likely to harbour disease-associated variants compared to arbitrary regions of the genome. Pathway analysis can improve power to uncover genetic factors relevant to disease because identifying

does not indicate which individual genes or variants are associated with disease, which remains an important question. To address this limitation, we describe a statistical framework that integrates enrichment analysis with disease-correlation tests for variants. We illustrate this approach in a case-control study for Crohn’s disease. We show that our approach uncovers disease-susceptibility genes that are not identified in conventional analyses of the same data.

Author Summary

Genome-wide association studies have helped locate genes that contribute to common diseases. The analysis of these studies is typically straightforward: systematically test each genetic variation in isolation whether it is correlated with susceptibility to disease. This approach often works well to identify commonly occurring variants with moderate effects on disease risk, but the effects of many variants are so small they fail to show statistically significant correlations. This is a concern because many common diseases are modulated by a large number of genetic factors with small effects on disease risk. An alternative strategy is to examine groups of variants, such as variants sharing a common biological pathway, and to test whether each group is enriched for disease correlations. This can be a more effective approach to identify genetic factors relevant to disease. However, it
the accumulation of small genetic effects acting in a common pathway is often easier than mapping the individual genes within the pathway that contribute to disease susceptibility.

A limitation of analyses that identify enriched pathways is that they do not provide feedback about associated variants within these pathways; identifying an enriched set of genes does not indicate which variants are associated with the disease, or even which genes harbour such variants. Yet discovery of associated variants and genes is a primary motivation for GWAS, and an important step toward understanding the biology of disease and, ultimately, developing effective medical therapies. In principle, identifying enriched pathways should help us locate variants associated with disease, because knowing that a variant lies in or near a gene in an enriched pathway makes it a better disease-association candidate. Therefore, prioritizing these variants—say, by slightly relaxing thresholds of significance—should yield a greater number of reproducible associations for a given rate of false positives. But it is unclear how to implement this in practice: to what extent can we relax significance thresholds while keeping the rate of false positives at an acceptable level?

To address this problem, we develop a model-based approach for joint analysis of pathways and genetic variants, in which we interpret enrichment as a model parameter. The enrichment parameter quantifies the increase in the probability that each variant in the pathway is associated with disease risk. By jointly analyzing variants and pathways, our method adjusts association evidence in light of estimated pathway enrichments—sometimes called pathway or gene prioritization—and, simultaneously, adjusts enrichment estimates to reflect evidence of associations in pathways.

Our approach builds on statistical methods for simultaneous mapping of genetic variants in GWAS. In contrast to single-marker regression approaches, these methods model susceptibility to disease by the combined effect of multiple variants, and use sparse multivariate regression techniques to fit multi-marker (i.e. polygenic) models to the data. By adopting a multi-marker disease model, estimating enrichment effectively reduces to counting, inside and outside each candidate pathway, variants associated with disease—more precisely, the variants that are included in the polygenic disease model. Our approach to combining multi-marker modeling with pathway analysis offers several benefits. First, compared to single-marker approaches, multi-marker modeling improves power to detect genetic associations. Second, unlike many pathway analysis methods that test for enrichment of significant SNPs or genes in a pathway, we have no need to select a significance threshold for p-values; instead, we use the association signal across all genes to assess enrichment. Third, by analyzing variants simultaneously, we avoid exaggerating evidence for enrichment from associated variants that are correlated with each other (i.e. in linkage disequilibrium), while still allowing multiple independent association signals near a gene to contribute evidence for enrichment. And fourth, quantifying enrichment within this framework naturally gives us feedback about associations within enriched pathways, potentially leading to discovery of novel genetic loci underlying disease.

Though we focus on incorporating pathways (and, more broadly, biologically related gene sets) into analysis of GWAS, our methods also apply to other types of genome annotations. In this respect, our work is related to other model-based approaches for estimating enrichment of genome-wide association signals across functionally related genomic regions, like transcription factor binding sites. One distinguishing feature of our approach is that we have the ability to test for enrichment, which is important for assessing which candidate pathways show the strongest support for enrichment. We assess support for enrichment by framing it as a model comparison problem. An advantage of this approach is that we can use the same approach to assess support for the enrichment of combinations of two or more pathways. This is useful, for example, if a pathway relevant to disease pathogenesis is ranked highly only after combining it with another pathway relevant to the disease.

Another feature that distinguishes our analysis is that we use multiple pathway databases in an attempt to interrogate pathways as comprehensively as possible—the more pathways we consider, the greater chance we have of drawing new connections between pathways, genes within these pathways, and complex disease. We demonstrate how using our approach to comprehensively interrogate pathways results in increased evidence for enrichment, and is robust to inclusion of a large number of irrelevant pathways. Our study includes ~3100 candidate pathways drawn from eight well-developed pathway databases available on the Web.

We demonstrate our approach in a detailed analysis of a GWAS for Crohn’s disease with about 440,000 single nucleotide polymorphisms (SNPs) genotyped in roughly 1700 cases and 3000 controls. This is a convenient study for gauging the benefits of our approach because genetic associations have already been published based on data from this study, and pathway analyses of these data have found evidence for enriched pathways. Our enrichment results highlight the role of cytokines that modulate immune responses in Crohn’s disease, and the IL-12 and IL-23 signaling pathways, which have been previously linked to the disease. By prioritizing variants within these enriched pathways, our method identifies disease-susceptibility candidates that are not deemed significant in conventional analyses of the same data, including the STAT3 gene, the IBD5 locus, and the major histocompatibility complex (MHC) class II genes. All these genetic associations have been independently confirmed in other studies, demonstrating that our methods have the potential to yield novel biological insights.

Overview of statistical analysis

Our approach builds on previous work that casts simultaneous analysis of genetic variants as a variable selection problem—the problem of deciding which variables (the genetic variants) to include in a multivariate regression of the phenotype. We begin with a method that assumes each variant is equally likely to be associated with the phenotype, and the major histocompatibility complex (MHC) class II genes. All these genetic associations have been independently confirmed in other studies, demonstrating that our methods have the potential to yield novel biological insights.
matrix $\mathbf{X}$ are observed minor allele counts $x_{ij} \in \{0, 1, 2\}$, or expectations of these counts estimated using genotype imputation \[57, 58\], for each of the $n$ samples and $p$ SNPs.

We assume an additive model of disease risk, in which the log-odds for disease is a linear combination of the minor allele counts:

$$
\log \left( \frac{p(y=1)}{p(y=0)} \right) = \beta_0 + \beta_1 \sum x_{ij} + \cdots + \beta_p x_{ip}.
$$

(1)

Under this additive model, $e^{\beta_j}$ is the odds ratio, the multiplicative increase in odds of disease for each copy of the minor allele at locus $j$. We do not consider dominant or recessive effects on disease risk, but it would be straightforward to include them; see \[59\]. This method is also easily adapted to quantitative traits by replacing (1) with a linear regression for $y$.

Although the log-odds for disease is expressed in (1) as a linear combination of all SNPs, we assume most SNPs $j$ have no effect on disease risk ($\beta_j = 0$). We refer to SNPs $j$ that have non-zero coefficients ($\beta_j \neq 0$) as being “included” in the multi-marker disease model. A SNP’s inclusion signals that it affects susceptibility to disease, or that is in linkage disequilibrium with other, possibly untyped, risk-conferring variants. Therefore, the main goal of the analysis is to compute, for each SNP $j$, the posterior inclusion probability, $\text{PIP}(j) \equiv p(\beta_j \neq 0 | \mathbf{X}, y)$. A high posterior inclusion probability is the analogue of a small $p$-value in a conventional single-marker analysis.

To obtain these posterior inclusion probabilities, we must first specify a prior. A standard assumption, and the assumption made in previous approaches \[58, 59\], is that SNPs are equally likely to be associated with the phenotype a priori; that is, $\pi_j \equiv p(\beta_j \neq 0)$ is the same for all SNPs.

To model enrichment of associations within a pathway, we modify this prior. Precisely, the prior inclusion probability for SNP $j$ depends on whether or not it is assigned to the enriched pathway:

$$
\log_{10} \left( \frac{\pi_j}{1 - \pi_j} \right) = \theta_0 + a_j \theta.
$$

(2)

The pathway indicators $a_j$ keep track of which SNPs are assigned to the enriched pathway: $a_j = 1$ when SNP $j$ is assigned to the enriched pathway, otherwise $a_j = 0$. In brief, a SNP is assigned to a pathway if it is near a gene in the pathway; see Methods. We refer to $\theta_0$ as the genome-wide log-odds, since it reflects the overall proportion of SNPs that are included in the multi-marker disease model. (More precisely, it is the proportion for SNPs not assigned to the pathway, but this is usually most SNPs.) We refer to $\theta$ as the enrichment parameter because it corresponds to the increase in probability (on the log-odds scale) that a SNP assigned to the pathway is included in the model. For example, $\theta_0 = -5$ and $\theta = 2$ indicates that 1 out of every 10,000 SNPs outside the pathway is included in the multi-marker model, but for SNPs assigned to the pathway, 1 out of every 100 are included. If $\theta = 0$, this reduces to the standard prior assumption made by previous methods. We expect $\theta$ to be zero, or close to zero, for most pathways.

To assess evidence for enrichment of a candidate pathway with indicators $a = (a_1, \ldots, a_p)$, we compute a Bayes factor \[62, 63\]:

$$
\text{BF}(a) = \frac{p(y | \mathbf{X}, a, \theta > 0)}{p(y | \mathbf{X}, \theta = 0)}.
$$

(3)

This Bayes factor (BF) is the ratio of likelihoods under two models, the model in which the candidate pathway is enriched for SNPs included in the multi-marker regression ($\theta > 0$), and the null model that no pathways are enriched ($\theta = 0$). A larger BF implies stronger evidence for enrichment. We compute each BF by averaging, or integrating, over the unknown parameters, and over multi-marker models with different combinations of SNPs, using appropriate prior distributions for $\theta_0$ and $\theta$ (see Methods).

We use the same approach to test for joint enrichment of multiple candidate pathways. We compute BF($a$) as before (eq. 3), except that we set $a_j$ to 1 whenever SNP $j$ is assigned to at least one of the pathways. In this case, $\theta$ represents the increased rate of associations among SNPs assigned to one or more of the pathways. This is equivalent to assuming that all enriched pathways have the same level of enrichment. It would be possible to relax this assumption, but at the cost of complicating the analysis, so we restrict ourselves to a single enrichment parameter.

To assess evidence for association of individual variants with the phenotype, we compute PIP($j$) for each variant $j$. These posterior probabilities depend on which pathways are enriched, and on the strength of enrichment $\theta$, because these factors affect the prior probabilities $\pi_j$, which in turn affect the posterior probabilities PIP($j$) following Bayes’ rule. (In practice, we account for uncertainty in $\theta_0$ and $\theta$ when calculating the posterior probabilities by averaging over $\theta_0$ and $\theta$; see Methods.) Since enrichment leads to higher prior inclusion probabilities for SNPs in the enriched pathway, an association that is not identified by a conventional genome-wide analysis, perhaps because the allele appears infrequently in the population, or because the nearby functional polymorphism has only a small effect on disease risk, may become a strong candidate in light of its presence in an enriched pathway. Because we estimate $\theta$ from the data, the extent to which we prioritize variants in enriched pathways is determined by the data. In this way, our framework integrates the problem of identifying enriched pathways with the problem of prioritizing variants within enriched pathways.

**Results**

We illustrate our methods through an extended example—analysis of genome-wide marker data from the WTCCC Crohn’s disease study \[51\]. After steps to ensure data quality, the data consist of ~440,000 SNPs genotyped for 1748 cases and 2938 controls. (See Methods for details.) Crohn’s disease is well suited to illustrating the benefits of pathway analysis because many pathways related to immune function and inflammatory response have been characterized. Additionally, genetic associations have been published based on data from this study \[51\], and have been replicated in a follow-up study \[62\]. Beyond these association analyses, enrichment analyses of these data have provided further support for links between Crohn’s disease and pathways related to adaptive and innate immunity \[17, 52, 55\].

We have three main goals in presenting this case study: first, to explain how to use and interpret the BF's, PIP's, and other statistics relevant to joint analysis of variants and pathways; second, to highlight the features, and limitations, of our approach; and third, to examine the hypothesis that we can gain additional insights into Crohn’s disease by...
reassessing the evidence for associations between variants and disease in light of pathway enrichment findings.

Our analysis proceeds in three stages. First, we compute a BF for each candidate pathway, and use these Bayes factors to rank the pathways. Second, based on this ranking, we assess evidence for models in which two or more pathways are enriched. Third, we investigate whether the most compelling pathway enrichments can help us locate Crohn’s disease associations beyond those identified in analyses that ignore information about pathways.

Bayes factors for enriched pathways

To rank the 3158 candidate pathways by their evidence for enrichment, we compute, for each pathway, a Bayes Factor that measures support for enrichment relative to the null hypothesis. All candidate pathways have been curated by domain experts, or are based on experimental evidence in other organisms and inferred via gene homology. To be as comprehensive as possible, we draw pathways from a variety of publicly accessible collections, and we include all pathways, even those that are unlikely to be relevant to Crohn’s disease based on current understanding of disease pathogenesis. To help make our results replicable, we document the pathway databases used, and the steps taken to compile gene sets from these pathway data. (See Table 4 for the list of pathway databases used; see Supplementary Results for statistics on gene sets and gene coverage from these databases; see Methods for retrieval and processing of pathway data, and assignment of SNPs to pathways.)

Many of the pathways in the databases are arranged hierarchically—we include all elements of the hierarchy in our analysis. Elements in upper levels of the hierarchy refer to groups of pathways with shared attributes or a common function. Some groups have a broad definition, such as “immune system” in Reactome, which includes pathways involved in adaptive and innate immune response. (Hereafter, we use the term “pathway” to refer to a set of biologically related genes.) Enrichment of a broad group of genes is unlikely to provide novel insights into disease pathogenesis. However, a key step in our analysis is to re-interrogate SNPs for association in light of inferred enrichments. Thus, enrichment of a broad physiological target like “immune system” can be useful if subsequent re-interrogation reveals associations that were not significant in a conventional analysis.

We find that the vast majority of pathways show little or no evidence for enrichment; of the 3518 candidate pathways, 2850 (90%) have BF < 1, and an additional 233 pathways (7%) have BFs between 1 and 10. Table 1 shows the 18 pathways with BF > 100. The cutoff at 100, as with any cutoff, is somewhat arbitrary. We discuss this issue and interpretation of the BFs below.

Several gene sets in Table 1 are subsets of one another (refer to Fig. 1 for relationships among these pathways in the Reactome and PID hierarchies). For example, “immune system” overlaps with eight other pathways in the table, including cytokine signaling. Several pathways appear in the table twice because the NCBI BioSystems and Pathway Commons databases sometimes disagree about the set of genes assigned to a pathway. These discrepancies can have a substantial impact on the results. For example, the BF for the Pathway Commons version of cytokine signaling is smaller than the BioSystems version by a factor of roughly 1000, due primarily to the lack of inclusion of NOD2 and MHC genes that contribute to the association signal. Conversely, the BF for the Pathway Commons version of IL-23 signaling is about 80 times larger than the BioSystems version because the former includes the NF-κB pathway, and this pathway contains several genes that contribute to the association signal, notably NOD2. (Inclusion of the NF-κB pathway is supported by experimental evidence.) These results illustrate the benefits of a comprehensive analysis that considers pathways from multiple sources.

Although IL-12 and IL-23 have distinct functions in regulating innate and adaptive immunity, these pathways implicate genes that contribute to immune response and differentiation of Th17 cells, even if many of the regulating activities previously ascribed to IL-12 are due to IL-23 instead. Additional findings from mouse models of inflammatory bowel disease support the role of IL-12 and IL-23. For example, IL-12 is also thought to be important for immune response and differentiation of Th17 cells, even if many of the regulating activities previously ascribed to IL-12 are due to IL-23 instead. Although IL-12 and IL-23 have distinct functions in regulation of T helper cells, their pathways have many cytokine and cytokine receptor components in common, so it may be difficult to tease apart their roles in disease solely by looking at enrichment in their gene sets; of the 66 genes
### Table 1. Pathways, and groups of related pathways, that exhibit the strongest evidence for enrichment of Crohn’s disease associations, as measured by their Bayes factors

| Enriched Pathway                                                                 | Database  | Number of Genes/SNPs | Bayes Factor | $\Theta_0$ | Enrichment $\bar{\Theta}$ |
|---------------------------------------------------------------------------------|-----------|-----------------------|--------------|------------|--------------------------|
| Cytokine signaling in immune system                                            | React. (BS) | $225/6711$ | $7.9 \times 10^{-5}$ | $-4.1$ | $1.96 (1.25-2.50)$ |
| IL-23-mediated signaling events                                                 | PID (PC) | $66/2218$ | $9.1 \times 10^{-5}$ | $-3.9$ | $2.01 (1.50-2.50)$ |
| IL-12-mediated signaling events                                                 | PID (PC) | $111/3641$ | $5.4 \times 10^{-3}$ | $-4.0$ | $1.90 (1.25-2.50)$ |
| Immune system                                                                  | React. (BS) | $755/20,959$ | $1.3 \times 10^{-3}$ | $-4.1$ | $1.44 (0.75-2.00)$ |
| Interferon signaling for immune system                                          | React. (BS) | $111/3678$ | $769$ | $-3.9$ | $1.70 (1.25-2.50)$ |
| Signaling by interleukins                                                       | React. (BS) | $73/2290$ | $700$ | $-3.8$ | $1.73 (1.25-2.25)$ |
| Interferon gamma signaling                                                      | React. (BS) | $529/15,074$ | $692$ | $-4.1$ | $1.49 (0.75-2.00)$ |
| Immunometabolism                                                                | React. (BS) | $111/2965$ | $491$ | $-3.8$ | $1.68 (1.25-2.25)$ |
| Cytokine signaling in immune system                                            | React. (PC) | $193/6194$ | $292$ | $-3.9$ | $1.55 (1.00-2.25)$ |
| Signaling events mediated by TC-PTP                                               | PID (PC) | $92/3044$ | $219$ | $-3.9$ | $1.62 (1.00-2.25)$ |
| TAK1 activates NF-$\kappa$B by phosphorylation and activation of IKBs complex  | React. (BS) | $24/884$ | $181$ | $-3.8$ | $2.00 (1.25-2.50)$ |
| Selective expression of chemokine receptors during T-cell polarization          | BioCarta  | $29/880$ | $139$ | $-3.8$ | $2.01 (1.25-2.75)$ |
| IL27-mediated signaling events                                                  | PID (PC, BS) | $26/796$ | $130$ | $-3.8$ | $2.00 (1.25-2.50)$ |
| IL-23-mediated signaling events                                                 | PID (BS) | $37/1252$ | $119$ | $-3.8$ | $1.89 (1.25-2.50)$ |
| CXCR4-mediated signaling events                                                 | PID (PC) | $190/6733$ | $118$ | $-3.9$ | $1.43 (0.75-2.00)$ |
| Activated TAK1 mediates p38 MAPK activation                                      | React. (BS) | $17/535$ | $116$ | $-3.8$ | $2.07 (1.25-2.75)$ |
| TCR signaling in naïve CD4$^+$ T cells                                          | PID (PC) | $133/4809$ | $103$ | $-3.9$ | $1.47 (0.75-2.00)$ |
| JNK phosphorylation and activation mediated by activated human TAK1            | React. (BS) | $16/559$ | $102$ | $-3.8$ | $2.03 (1.25-2.75)$ |

The table includes all gene sets with BF > 100. Abbreviations used in this table: React. = Reactome [63], PID = NCI Nature Pathway Interaction Database [64], BS = NCBI BioSystems [65], PC = Pathway Commons [66]; $\Theta_0$ = posterior mean of genome-wide log-odds, $\bar{\Theta}$ = posterior mean of enrichment (and 95% credible interval) given that pathway is enriched. The credible interval is the smallest interval about the posterior mean that contains $\theta$ with 95% posterior probability. It is calculated to the nearest 0.25 using a numerical approximation (see Supplementary Methods). Note that these numbers may not be reproduced exactly in an independent analysis using the same method, due to stochasticity in our approximate computations. However, only slight deviations from these numbers are expected.

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assigned to the IL-23 pathway, 54 are assigned to the IL-12 pathway (Pathway Commons version).

Previous findings from genome-wide association studies have linked autophagy genes ATG16L1 and IRGM to Crohn’s disease [51, 73, 81]. Our pathway analysis does not provide additional support for autophagy in Crohn’s disease because pathways reflecting current models of autophagy [53, 55] have not yet been incorporated, to our knowledge, into any of the publicly available pathway databases.

Many of the pathways listed in Table 1 are related to those identified in previous pathway analyses of Crohn’s disease [51, 73, 81], including Jak-STAT signaling [53] and T cell receptor signaling [55]. Other pathways highlighted in previous analyses show some evidence for enrichment in our analysis, but these are eclipsed by much stronger enrichment signals (Table 1). For example, Wang et al [55] obtain the most evidence for enrichment of “IL12 and Stat4 dependent signaling in Th1 development” ($p$-value $= 8 \times 10^{-5}$, FDR $= 0.045$) based on enrichment analysis of BioCarta, KEGG and Gene Ontology [53] gene sets, whereas this pathway showed only modest evidence for enrichment in our analysis (BF = 18) compared to the pathways in Table 1. (Below, we obtain greater support for enrichment of this pathway when combined with cytokine signaling genes.) Moreover, all except one of the pathways in Table 1 are from databases that were not included in [53]. These results illustrate the benefits of a comprehensive search for enrichment across multiple pathway databases.

Given that enrichment analyses typically proceed by computing $p$-values and assessing “significance,” one may reasonably ask whether the BFs in Table 1 represent “significant” evidence for enrichment. Specifying an appropriate threshold for a BF to be considered significant, however, is context-dependent, and subjective. This is because the posterior odds for a pathway being enriched, relative to the null hypothesis that no pathways are enriched, is equal to the Bayes Factor times the prior odds for enrichment. The posterior odds for each pathway depends on how plausible it is, which is quantified by the prior probability of enrichment [83, 84]. In practice, however, significance thresholds of 0.05 or 0.01 are often used without attending to this concern.) Nonetheless, we can make the following observations. First, if we are willing to assume the pathways in Table 1 are all equally plausible candidates for enrichment a priori, then the ratio of the BFs indicates the relative support for the enrichment hypotheses. For example, if we are forced to choose between enrichment of “cytokine signaling in immune system” and “IL-23-mediated signaling events,” the data overwhelmingly favour the former by a factor of $\frac{92/3044}{193/6194} \approx 87$. Second, even under a “conservative” prior for enrichment in which we expect one pathway to be enriched among the 3158 candidates, corresponding to a prior odds of 1/3158, the top
three pathways have BFs that are large enough (greater than 3158) to support enrichment.

### Assessing combinations of pathways for enrichment

The initial ranking (Table 1) suggests that enrichment of a group of pathways, cytokine signaling in immune system, offers a better fit to the pattern of Crohn’s disease associations than any one pathway. But the question remains whether some other combination of pathways offers a better fit. A benefit of our approach is that we can directly compare support for enrichment of different combinations of pathways by comparing their BFs (assuming the same prior for these hypotheses). This is because the ratio BF($a$)/BF($a^∗$) is the same as the Bayes factor that compares support for the enrichment model encoded by $a$ versus the model encoded by $a^∗$. (By contrast, it is harder to make such comparisons using $p$-values. For example, if $p$ is the $p$-value for testing hypothesis $a$ against the null, and $p^∗$ is the $p$-value for testing $a^∗$ against the null, it is not clear how to compare support for $a$ and $a^∗$.)

We begin by computing BFs to quantify support for enrichment of pairs of pathways. Since it is impractical to consider all pairs, we tackle this in a “greedy” fashion by selecting combinations of pathways based on the initial ranking. Our strategy is to select pathways with the largest BFs (here we take IL-23, IL-12 and cytokine signaling), and we consider each of these in combination with pathways from a larger set of candidates (here we use the 72 pathways with BF > 10). This greedy heuristic makes it feasible to evaluate many combinations of pathways that could plausibly be jointly enriched, though since it does not consider all combinations, there is the risk of overlooking a combination that provides a better fit to the pattern of associations. In total, we compute BFs for 219 pairs of pathways, which includes 3 combinations of IL-23, IL-12 and cytokine signaling, and 216 = 3 × 72 combinations of IL-23, IL-12 or cytokine signaling with another pathway.

Table 2 lists all models with two enriched pathways that have BF > 10^8. As before, all these pathways are related to innate and adaptive immune processes. Three of the BioCarta pathways appearing in this table, including the IL-12 and Stat4 dependent signaling pathway highlighted in the enrichment analysis of Carbonetto and Stephens (2011) did not originally show up in Table 1 because their BFs were less than 100. The largest BF, for enrichment of IL-23 and cytokine signaling, is unsurprising in light of the initial ranking, except that this version of the IL-23 pathway does not include the NF-κB pathway, which suggests that the NF-κB pathway does not contribute additional evidence for enrichment once we account for enrichment of cytokine signaling genes (14 of the 35 genes in the NF-κB pathway, including NOD2, are also members of cytokine signaling). Among hypotheses that do not involve cytokine signaling, the largest BF is 5.1 × 10^8, corresponding to a model in which IL-23 signaling and interferon gamma signaling are enriched.

The top BF in Table 2 is ~700 times greater than the largest BF in Table 1. This suggests that the best model with two enriched pathways provides a much better fit to the data than the best model with any one enriched pathway. However, to properly interpret this result we must weigh this increase in the BF against the relative prior plausibility of the models, which is difficult to quantify. A naive argument using a “conservative” prior for any pair of pathways being enriched might suggest a prior odds of (1/3158)^2, based on the conservative prior for a single pathway we discussed above. This prior would make a 700-fold increase in the BF appear to be relatively insignificant.

However, this argument not only depends on the prior, which may be overly conservative, but also assumes independence of enriched pathways, which seems unwise considering that many pathways mentioned here have related roles in immunity; a priori, one might expect that a pathway is more likely to be enriched when a biologically related pathway is enriched. With this in mind, we interpret Table 2 as providing substantial, if short of compelling, support for the hypothesis that two pathways are enriched for Crohn’s disease associations. Perhaps a more important question is whether these findings lead to identification of additional loci affecting susceptibility to Crohn’s disease, a question we address in the next section.

For completeness, we extend the analysis to models with three enriched pathways. Again, following a greedy strat-
Fig. 1. Scatterplots showing $P_1$, the posterior probability that each genomic segment contains Crohn’s disease associations, given different hypotheses about enriched pathways. Each point corresponds to a segment of the genome containing 50 SNPs. Some of the segments are labeled by representative candidate genes. In panel A, genes assigned to “cytokine signaling in immune system” are written in bold.

Associations informed by enriched pathways

Now we examine how the pathway enrichment findings can help us identify additional genetic associations. The intuition is that, having established that variants near genes in a pathway are more likely to be associated with the phenotype, it is reasonable to up-weight, or prioritize, these SNPs in the statistical analysis. Our model-based framework achieves this by estimating an enrichment parameter corresponding to the increased prior probability that SNPs assigned to the pathway are associated with the phenotype. This prior probability in turn raises the posterior probability of association for these SNPs. This ability to reassess the evidence for association of individual loci in light of enriched pathways is an important feature of our model-based approach to pathway analysis, as identifying individual disease-susceptibility loci is potentially more informative than observing that a pathway is enriched for disease associations. This is particularly the case for broad pathways, such as cytokine signaling in immune system, which contains 225 genes, as only a small proportion of these genes may actually harbour genetic variants that affect Crohn’s disease risk.

In simultaneous analysis of genetic variants based on Bayesian variable selection, it is preferable, at least initially, to assess evidence for associations across genomic regions, rather than for individual SNPs. This is because when SNPs are correlated with one another the association signal can be spread across these SNPs, diluting the signal at any given SNP. Therefore, we divide the genome into overlapping segments of 50 SNPs, with an overlap of 25 SNPs between neighbouring segments. For each segment, we compute $P_1$, the posterior probability that at least one SNP in the segment is included in the multi-marker disease model. We use segments with an equal number of SNPs so that, under the null hypothesis of no enrichment, the prior probability that at least one SNP is included is the same for every segment. Since a segment spans, on average, 307 kb of the genome (98% of segments are between 100 kb and 1 Mb long), calculating $P_1$ for these segments provides only a low-resolution map of genetic risk factors for Crohn’s disease. Still, this resolution suffices for the objectives of this case study. In other applications, one could increase the resolution by calculating $P_1$ for individual SNPs within selected regions. Hereafter, we use $P_1$ to denote the posterior probability that at least $n$ SNPs are included.

First, we compare Crohn’s disease associations identified under the null hypothesis that no pathways are enriched with associations identified under the model in which a single pathway, cytokine signaling in immune system, is enriched. Figure 1A shows how the evidence for association in each segment ($P_1$) changes once we account for enrichment of cytokine signaling genes. As expected, many segments (corresponding to points above the diagonal in the scatterplot) show increased evidence for association under the model in which cytokine signaling is enriched. These segments contain SNPs assigned to the enriched pathway. Segments corresponding to points on the diagonal show no change in support for association, and these are segments that do not contain SNPs assigned to the enriched pathway. Although it is not clear from the figure due to over-plotting, most segments lie near the bottom-left corner; when cy-
Table 3. Selected regions of the genome with strong evidence for Crohn’s disease risk factors given that two pathways are enriched for Crohn’s disease associations

| chr. | critical region (Mb) | P1 | P2 | candidate gene(s) | SNP | MAF |
|------|---------------------|----|----|------------------|-----|-----|
| 1p31 | 67.30–67.48         | 1.00 | 1.00 | 0.06           | 0.48 |     |
| 2q37 | 233.92–234.27       | 1.00 | 1.00 | 0.01           | 0.22 | A1G16L1 |
| 5p13 | 40.32–40.56         | 1.00 | 1.00 | 0.42           | 0.42 | PTEG4A |
| 5q23 | 129.54–132.04       | 0.18 | 0.86 | 0.02           | 0.29 | MHC class II |
| *7 | 6p21 | 32.3–32.92         | 0.49 | 0.98 | 0.02           | 0.31 | HMC class II |
| 10q21 | 64.0–64.43      | 0.96 | 0.96 | 0.03           | 0.03 | ZNF365 |
| 10q24 | 101.26–101.32      | 0.95 | 0.95 | 0.01           | 0.04 | NKX2-3 |
| 16q12 | 49.0–49.49       | 1.00 | 1.00 | 0.11           | 0.77 | NOD2 |
| *17q21 | 37.5–38.3        | 0.12 | 0.87 | 0.01           | 0.21 | STAT3 |
| 18p11 | 12.76–12.91      | 0.94 | 1.00 | 0.01           | 0.20 | PTPN2 |

For every region in this table, there is at least a 0.8 probability that one or more SNPs in the region are included in the multi-marker disease model ($P_1 ≥ 0.8$) given the hypothesis that two pathways are enriched. Rows marked with an asterisk (*) are selected only after accounting for enriched pathways. Table columns from left to right are: (1) chromosomal locus; (2) region most likely containing the risk-conferring variant(s), in Megabases (Mb); (3) posterior probability that one or more SNPs in the region are included in the model under the null hypothesis; (4) under the alternative hypothesis that two pathways are enriched; (5) posterior probability that two or more SNPs are included under the null, and (6) under the alternative; (7) frequency of minor allele for that SNP in cases; and (10) in controls. The “critical region” at each locus is estimated by inspecting single-SNP BFs and, and bounding the region by areas of high recombination rate, inferred using data from Phase I, release 16a of the HapMap study, and visualized in the UCSC Genome Browser [84]. All SNP information and genomic positions are based on human genome assembly 17 (NCBI build 35).

tokine signaling is enriched, 17,261 out of 17,668 segments across the genome (97.7%) have $P_1 ≤ 0.1$.

Points in the top-right corner of Fig. 1A correspond to regions with strong evidence for association even without the benefit of feedback from pathway enrichment. Genes IL23R, PTEG4A, ZNF365, NKX2-3 and A1G16L1 are not involved in cytokine signaling, nor are any nearby genes, so the PIPs of SNPs near these genes are unaffected by the hypothesis that cytokine signaling is enriched. NOD2 (also known as CARD15) and PTPN2 are cytokine signaling genes, so these associations contribute to the evidence for enrichment of this pathway, but because they already show strong support for association without enrichment ($P_1$ is close to 1 under the null hypothesis), these associations are not greatly affected by enrichment. Reassuringly, these results recapitulate the strongest associations reported in the original study (Table 3 in [51]) with trend $p$-values less than $4 \times 10^{-8}$, or those with additive BFs greater than $10^{4}$. These results have also been replicated in a follow-up study [62], and have been confirmed in meta-analyses with large combined sample sizes [6, 7]. Two additional regions at 3p21 and 5q33 are reported as associations in the original study [51], although with trend-test $p$-values exceeding $5 \times 10^{-8}$. These Crohn’s disease associations are replicated elsewhere [6, 7, 62], but show only modest evidence for association in our analysis; these regions are not annotated to the cytokine signaling pathway, and the largest $P_1$ for segments at these regions are 0.19 and 0.21, respectively.

Points near the top-left corner of Fig. 1A correspond to regions of the genome that show strong support for association only after accounting for enrichment of cytokine signaling. Three additional regions stand out: the MHC class II region, previously identified as a region showing moderate evidence of association [51]; the IBD5 locus at 5q31, which contains several candidate genes; and a region at position 17q21 near gene STAT3. Other genome-wide studies and meta-analyses independently support Crohn’s disease associations at these loci [6, 7, 61]. (See Supplementary Results for further details on these loci.) In addition, two loci at 16q24 and 10q22 near genes IRF8 and CAMK2G show moderate support for association under the enrichment hypothesis; $P_1$ is 0.49 and 0.46, respectively. Neither of these loci have been identified as being significant associations in other Crohn’s disease studies. However, the association at locus 16q24, 84.45–84.46 Mb near IRF8 is potentially interesting, as it has previously been identified in genome-wide studies of two other autoimmune diseases, multiple sclerosis [92] and systemic sclerosis [93]. This gene belongs to a family of transcription factors that regulate responses to type 1 interferons (IFN-α and IFN-β), and these interferons are known play critical roles in modulating inflammatory and immune responses to pathogens [72].

Next we investigate whether allowing for two enriched pathways reveals any further associations. (See Methods for how $P_1$ is computed by averaging over different models with two enriched pathways.) Figure 1B shows that allowing for enrichment of two pathways does not yield compelling support for genetic associations beyond those revealed by enrichment of cytokine signaling. However, the segment with the greatest increase in $P_1$, from 0.04 to 0.53, is at 158.4–159.1 Mb on chromosome 5, near gene IL12B. This locus was reported as a Crohn’s disease association in [6], and was later confirmed in [7]. IL12B is also associated with other autoimmune diseases, including ulcerative colitis [94, 95] and psoriasis [96]. Note that all pathways listed in Table 2 except cytokine signaling implicate IL12B, so the association signal at this locus presumably contributes to evidence for enrichment of these pathways.

Table 3 summarizes the Crohn’s disease associations that are strongly supported by our analysis. Of the 10 regions listed in this table, 3 are revealed only after prioritizing SNPs in enriched pathways. Each row in the ta-
ble shows the SNP within the critical region that has the largest PIP. In most cases, since only a portion of all commonly occurring SNPs are included in the study, this SNP is most likely in linkage disequilibrium with the causal variant rather than being causal itself. We also show in this table, for each selected region, evidence for multiple independent risk factors, indicated by $P_2 \approx 0.77$, a prediction that coincides with a previous study [97].

The large number of points approaching the middle of the $y$-axis in Fig. 2A suggests that many other gene variants involved in cytokine signaling may contribute to Crohn’s disease risk. In fact, the estimates $\theta_j = -4.11$ and $\theta = 1.96$ given in Table 1 imply that roughly 1 out of every 140 SNPs in the pathway are expected to be independent associations, for a total of about 47 independent risk factors for Crohn’s disease hidden among “cytokine signaling in immune system” genes. Our analysis has only identified a few of these genes, which suggests that many more associations in this pathway remain to be discovered. This prediction coincides to some extent with a recent meta-analysis of Crohn’s disease association studies [7], as an additional 7 cytokine signaling genes—IL1R1, IP6K2, JAK2, IL2RA, TYK2, MAPK1, and MAP3K7IP1—overlap with the susceptibility loci identified in this meta-analysis.

**Sensitivity of pathway ranking to prior distribution of odds ratios**

Our approach requires specification of a prior distribution for the odds ratios (see Methods). We assume a prior in which the log-odds ratios (i.e. the coefficients $\beta_j$ in the additive model of disease risk) are normally distributed with mean zero and standard deviation $\sigma_\beta = 0.1$. This choice is based on odds ratios reported in published genome-wide association studies (see Methods). One concern is that slightly smaller or slightly larger settings of $\sigma_\beta$ could also be justified, and these choices could produce different results. Associations are unlikely to accumulate at a greater rate in pathways that are not related to the disease, even associations with small effects on disease risk, so we predict that the ranking of pathway enrichments is largely robust to the choice of $\sigma_\beta$. Here we verify this claim. We assess the sensitivity of our results to $\sigma_\beta$ by recomputing the BF$s$ for all candidate pathways with prior choices that favor slightly smaller ($\sigma_\beta = 0.06, 0.08$) and slightly larger coefficients ($\sigma_\beta = 0.15, 0.2$).

Fig. 2 shows that smaller settings of $\sigma_\beta$ yield substantially more support for enrichment of disease-related pathways, as expected. But the pathways with the largest BFs are IL-23, IL-12, and cytokine signaling regardless of the choice of $\sigma_\beta$. In the Supplementary Results, we show that the BFs for most of the other 3158 candidate pathways do not change substantially at different settings of $\sigma_\beta$. In summary, we conclude that the pathway-level findings in this Crohn’s disease study are largely robust to priors that are not substantially different from $\sigma_\beta = 0.1$.

**Discussion**

Pathway analysis for genome-wide association studies has been as advertised as a way to overcome some of the limitations of conventional approaches to identifying genetic factors underlying polygenic traits. Motivated by the observation that it is easier, in principle, to identify associations within an enriched pathway, we developed a model-based approach for simultaneously estimating enrichment and prioritizing variants in enriched pathways. We investigated the merits and limitations of this approach in a GWAS for Crohn’s disease. In this case study, we interrogated over 3,000 candidate pathways from several pathway databases, and confirmed the importance of the IL-12 and IL-23 pathways in Crohn’s disease pathogenesis and, more broadly, the role of cytokines that mediate immune responses. By prioritizing variants within the enriched pathways identified in our analysis, we established strong support for disease susceptibility loci beyond what are revealed by a conventional analysis that ignores this pathway information. This suggests that applying our methods to larger samples may reveal genetic loci that have not yet been identified as risk factors for Crohn’s disease and other common diseases. Moreover, leveraging knowledge about variants that modulate expression of genes (eQTLs) may also lead to stronger evidence for pathway enrichments, and for associations within these pathways.

Our approach to modeling enrichment was built on large-scale sparse regression methods that have been applied to other problems in statistics and genetics. The key idea behind our approach was to introduce a parameter, $\theta$, that quantifies enrichment—precisely, the increase in the probability that a SNP assigned to a pathway is included in a polygenic model of the phenotype. Given the generality of this approach, our method could be useful for problems outside genetic association studies. One caveat is that some of the approximations we used—approximations which help scale the computations to hundreds of thousands of SNPs and thousands of candidate pathways (see Supplementary Methods)—may not be appropriate in some settings.

One attractive feature of our approach, which we illustrated in the Crohn’s disease case study, is that it can be
used to assess how well the data support enrichment of different combinations of multiple pathways. Examining combinations of pathways for enrichment may highlight interesting pathways that would not otherwise be highly ranked. An example of this was the “IL-12 and Stat4 dependent signaling” pathway, which showed little support for enrichment on its own (BF = 18), but became more interesting when considered jointly for enrichment with cytokine signaling genes (BF = 2.4 × 10^3).

In contrast to many pathway analysis methods, we modeled pathway enrichment at the level of variants, rather than genes. While there are arguments for both approaches, a feature of the variant-based approach is that, when there are multiple independent association signals near a gene, all these signals contribute to the evidence for enrichment of pathways containing this gene.

The Crohn’s disease study was aimed at identifying common variants associated with disease, but there is growing interest in using exome and whole-genome sequencing to investigate the contribution of rare variation to complex diseases and traits [59, 103]. The computational complexity of our method grows linearly with the number of SNPs, so it should scale well to the large amount of data generated by high-throughput sequencing. Since detecting associations with individual rare variants is a hard problem [101, 102], pathway-based analysis approaches such as ours that aggregate association signals across sets of genes may play an important role in analysis of these studies.

Currently, a drawback to our approach is that the prior variance of additive effects on disease risk must be chosen beforehand. We based our choice on the distribution of odds ratios reported in published genome-wide association studies, and checked that the rankings of enriched pathways were robust to different prior choices. Ideally, we would estimate this parameter from the data instead, but, as we discussed in Methods, we found that this did not work well for the Crohn’s disease data. In our estimation, the root of the problem is that the log-odds ratios are not normally distributed (currently, we assume that non-zero effects follow a normal distribution). One possible solution would be to use a more flexible distribution for the effect sizes, such as a mixture of two or more normals, but we have not investigated this direction as it raises a number of questions regarding modeling and computation. Despite this issue, we believe that we have presented a useful framework for identifying enriched pathways and genetic associations underlying these pathways.

### Method

#### Samples

Our analysis is based on genome-wide marker data from a case-control study with 1748 subjects affected by Crohn’s disease, and 2938 control subjects. The controls come from two groups: 1480 individuals from the 1958 Birth Cohort, and 1458 individuals from the UK Blood Services cohort. All subjects are from Great Britain, and of self-described European descent. Genetic associations from this study were first reported in [51].

All study subjects were genotyped for ~500,000 SNPs using a commercial version of the Affymetrix GeneChip 500K platform. We apply quality control filters as described in [51], and remove SNPs that exhibit no variation in the sample. We discarded two additional SNPs, rs1914328 on chromosome 8 and rs6601764 on chromosome 10, because they show some evidence for association (single-SNP BFs of 10^{−5.5} and 10^{−6}, respectively), but we cannot rule out the possibility of genotyping errors as these associations are not supported by nearby SNPs (none of the nearby SNPs have single-SNP BF's greater than 40). After removing these two SNPs, we end up with 442,001 SNPs on autosomal chromosomes. We estimate missing genotypes at these SNPs using the mean posterior minor allele count from BIMBAM [58, 103], with SNP data from Phase II of the International HapMap Consortium project [104]. To be consistent with the original analysis, refSNP identifiers and locations of SNPs are based on human genome reference assembly 17 (NCBI build 35).

#### Population stratification

Analysis of pathways should, in principle, be robust to population stratification because spurious associations that arise from population structure are unlikely to accumulate at a greater rate in the pathway—recall, we define the enrichment parameter as the increase in proportion of variants associated with disease risk relative to the proportion genome-wide. However, population stratification could still produce individual false positive associations, either inside or outside enriched pathways, so in general one should account for this in the analysis [40, 103, 105]. For the Crohn’s disease study, the original report [51] and subsequent analyses [40, 106] affirm that cryptic population structure does not have a substantive impact on the analysis. Thus we do not attempt to correct for population structure in our analysis.

#### Pathways, and assignment of SNPs to pathways

We aim for a comprehensive evaluation of pathways accessible on the Web in standard, computer-readable formats [40, 54, 116]. Since the results hinge on the quality of the pathways used in our analysis, we restrict the analysis to curated, peer-reviewed pathways based on experimental evidence, and pathways inferred via gene homology. We draw candidate pathways from the collections listed in Table 3 (see Supplementary Methods for details). KEGG and HumanCyc are primarily databases of metabolic pathways, and are unlikely to be relevant to Crohn’s disease, but we include them for completeness.

Since we combine pathways from different sources, we encounter pathways with inconsistent definitions [117, 118]. There is no single explanation for the lack of consensus in pathway definitions, and we have no reason to prefer one definition over another, so we include multiple versions of a pathway in our analysis. Many of the pathways in these databases are arranged hierarchically; we incorporate all elements of the hierarchy into our analysis. We treat each candidate pathway as a set of genes, ignoring details such as molecules involved in biochemical reactions, and cellular locations of these reactions. From 3788 total pathways (Table 3), we obtain 3158 unique gene sets. With these pathways we achieve ~39% gene coverage (see Supplementary Results).

Based on findings that the majority of variants modulating gene expression lie within 100 kb of the gene’s tran-
For a 20 kb window \[52, 55\] based on findings that expression QTLs are rarely more than 20 kb from the gene \[119–121\], we assign a SNP to a gene if it is within 100 kb of the transcribed region. Others have opted to consider only SNPs that are in cis relationship to the transcriptional start site. Within this window, we assign a SNP to a gene if its expression is regulated by that gene. We also assign a SNP to a gene if it is in linkage disequilibrium with one another, leaving only one marker for each independent association—an association that signals a variant contributing to disease risk independently of other risk-conferring variants.

### Statistical analysis

The Bayesian variable selection approach to simultaneous interrogation of SNPs involves fitting the multi-marker disease model to the data using different combinations of SNPs. Fitting all markers simultaneously weeds out multiple associations at markers that are in linkage disequilibrium with one another, leaving only one marker for each independent association—an association that signals a variant contributing to disease risk independently of other risk-conferring variants.

#### Likelihood

The likelihood specifies the probability of observing the phenotype observations \(y\) given the genotypes \(X\), the intercept \(\beta_0\), and the regression coefficients \(\beta = (\beta_1, \ldots, \beta_m)\). From the additive model for the log-odds of disease (eq. 1),

\[
p_i = \psi(\beta_0 + \sum_{j=1}^m x_{ij} \beta_j) = \frac{\exp(\beta_0 + \sum_{j=1}^m x_{ij} \beta_j)}{1 + \exp(\beta_0 + \sum_{j=1}^m x_{ij} \beta_j)}
\]

where \(\psi(x) = 1/(1+e^{-x})\) is the sigmoid function. Assuming independence of the observations \(y_i\), the likelihood is

\[
p(y | X, \beta_0, \beta) = \prod_{i=1}^n p_i^{y_i} (1 - p_i)^{1-y_i}. \tag{4}
\]

#### Priors

Next we specify prior distributions for the genome-wide log-odds \(\theta_0\), the enrichment parameter \(\theta\), the intercept \(\beta_0\), and the coefficients \(\beta_j\) of SNPs included in the multi-marker model of disease risk.

Since inferences strongly depend on \(\theta_0\), and since \(\theta_0\) is unknown and will be different for each setting, we estimate this parameter from the data rather than fix it a priori. Following \[39, 57\], we assign a uniform prior to \(\theta_0\). We restrict \(\theta_0\) to \([-6, 2]\), so as few as 0 and as many as 4400 SNPs are expected to be included a priori.

We place a uniform prior on \(\theta\), restricted to \([0, 3]\). This prior permits a wide range of enrichments because, in our view, enrichments greater than a thousand-fold are unlikely to occur. Note that we do not allow negative enrichments; that is, we do not consider pathways that are underrepresented for associations with the phenotype. Negative enrichments could potentially be useful in other scenarios, but for most genome-wide association studies where there are generally few significant associations to begin with, negative pathway enrichments are difficult to find and are unlikely to have a useful interpretation.

For the prior on the non-zero coefficients \(\beta_j\), we follow a standard practice that assumes they are i.i.d. normal with zero mean and standard deviation \(\sigma_j\). Ordinarily, to combat sensitivity of the results to the choice of \(\sigma_a\), we would place a prior on \(\sigma_a\) and integrate over this parameter to let the data drive selection of \(\sigma_a\). This approach is taken in \[38, 57\]. But in our case, we find that the heterogeneity of the odds ratios in Crohn’s disease presents a problem: although we expect most odds ratios for a common disease—and specifically odds ratios in a pathway relevant to disease pathogenesis—to be close to 1, the odds ratios corresponding to the strongest Crohn’s disease associations drive estimates of \(\sigma_a\) toward larger values, and a normal distribution that puts too little weight on modest odds ratios. One possible strategy would be to redo the analysis after removing associated regions with the largest odds ratios, but this is an unattractive solution because SNPs with large odds ratios would not contribute to the evidence for enrichment. Instead, we fix \(\sigma_a\), grounding the choice on typical odds ratios reported in published genome-wide association studies, and we assess the robustness of our findings to this choice. (There is the potential for more principled solutions; see Discussion.) Our choice is \(\sigma_a = 0.1\), which favours odds ratios close to 1 (95% of the odds ratios lie between 0.82 and 1.22 a priori), while being large enough to capture a significant fraction of the odds ratios for common alleles reported in genome-wide association studies of complex disease traits. According to a recent review \[1\], approximately 40% of estimated odds ratios are between 1.1 and 1.2, and an additional 10% of odds ratios are smaller than 1.1. This prior also closely corresponds to a survey of

| database                      | refs. | download location         | #pathways |
|-------------------------------|-------|----------------------------|-----------|
| BioCarta                      | [www.openbioinformatics.org/gene] | Pathway Commons | 298       |
| Cancer Cell Map               |       | NCBI BioSystems           | 54        |
| HumanCyc                      | [109, 110] | Pathway Commons | 224       |
| Kyoto Encyclopedia of Genes and Genomes (KEGG) | 111 | NCBI BioSystems | 399       |
| NCI Nature Pathway Interaction Database (PID) | 64 | NCBI BioSystems | 186       |
| PANTHER                       | [112, 113] | Pathway Commons | 179       |
| Reactome                      | 63    | NCBI BioSystems           | 103       |
| WikiPathways                  | [114, 115] | NCBI BioSystems | 147       |

From these 3788 pathways, we obtain 3158 unique gene sets.
odds ratios reported in genetic association studies of common diseases [124]. Since there may be justification for a slightly smaller or slightly larger $\sigma_\alpha$, we also try different values for $\sigma_\alpha$, and examine how these choices affect the ranking of enriched pathways (see Results).

To complete the probability model, we assign an improper uniform prior to the intercept, $\beta_0$. In general, one must be careful with use of improper priors in Bayesian variable selection because they can result in improper posteriors. A sufficient condition for a proper posterior, and a well-defined BF, with logistic regression (eq. 1) is that the maximum likelihood estimator of $\beta$ conditioned on which variables are included in the model, and on the other model parameters, is unique and finite [123]. Unfortunately, this condition is difficult to check exhaustively. But we can at least guarantee that the posterior is proper under the variational approximation (see Supplementary Methods) so long as the coordinate ascent steps converge to a unique solution.

Bayes factors and posterior odds

Ideally, we would assess evidence for an enrichment model by computing the posterior probability for that model. But computing these posterior probabilities is impractical for several reasons, one being that it would involve computations for a large number of combinations of pathways. Instead, we compute a Bayes factor [3] for each candidate pathway, or combination of pathways, then we weigh the Bayes factor against the prior odds to obtain the posterior odds for the enrichment model:

\[
\frac{p(\theta > 0 | X, y, a)}{p(\theta = 0 | X, y, a)} = \frac{p(y | X, a, \theta > 0)}{p(y | X, a, \theta = 0)} \times \frac{p(\theta > 0)}{p(\theta = 0)}. \tag{5}
\]

In our pathway enrichment results (Tables 1 and 2), we report BFs rather than posterior odds. Although our results would be more straightforward to interpret had we provided posterior odds instead of BFs, posterior odds are easily obtained from BFs, and reporting BFs offers the reader flexibility to judge the evidence based on his or her own prior. In the results, we illustrate how to gauge support for each enrichment hypothesis by weighing against a “conservative” prior. However, the reader may have reason to choose a different prior, such as one that favours certain pathways above others.

To allow for uncertainty in $\theta_0$ and $\theta$ when evaluating the BFs, the likelihood under the enrichment hypothesis ($\theta > 0$) and the likelihood under the null ($\theta = 0$) are each expressed as an average over possible assignments to $\theta_0$ and $\theta$:

\[
BF(a) = \frac{\int p(y | X, a, \theta_0, \theta) p(\theta_0) p(\theta) d\theta d\theta_0}{\int p(y | X, a, \theta_0, \theta = 0) p(\theta_0) d\theta_0}. \tag{6}
\]

Each instance of $p(y | X, a, \theta_0, \theta)$ in \ref{eq:5} expands as an average over possible assignments to the intercept $\beta_0$ and regression coefficients $\beta$:

\[
p(y | X, a, \theta_0, \theta) = \int \prod_{j=1}^{p} p(\beta_j | a_j, \theta_0, \theta) d\beta_0 d\beta. \tag{7}
\]

Factor $p(\beta_j | a_j, \theta_0, \theta) = \pi_j N(0, \sigma^2_\beta) + (1 - \pi_j) \delta_0$ is the “spike and slab” prior [122, 123], in which $\pi_j = p(\beta_j \neq 0)$ is determined according to \ref{eq:9}. Here, $\delta_0$ denotes the delta mass, or “spike”, at zero, $N(\mu, \sigma^2)$ is the normal density with mean $\mu$ and variance $\sigma^2$, $p(\beta_0)$ is the (improper) uniform prior, and $p(y | X, \beta_0, \beta)$ is the logistic regression likelihood [11]. Computation of Bayes factors is described in Supplementary Methods.

Posterior inclusion probabilities and other statistics

Here we define PIPs and other posterior quantities used in our analysis for the case when a pathway, or combination of pathways, is enriched. Posterior statistics under the null hypothesis are obtained by setting $\theta = 0$.

Like the BFs, the PIPs are obtained by averaging over $\theta_0$ and $\theta$. Taking $\mathcal{D} = \{X, y, a\}$ as shorthand for all the data, we have

\[
PIP(j) = p(\beta_j \neq 0 | \mathcal{D}) = \int p(\beta_j \neq 0 | \mathcal{D}, \theta_0, \theta) p(\theta_0, \theta | \mathcal{D}) d\theta_0 d\theta. \tag{8}
\]

To identify regions of the genome associated with disease risk, we calculate, for each region, the posterior probability that at least one SNP in the region is included in the multi-marker disease model (see Results for an explanation). Let $S = n$ represent the event that exactly $n$ SNPs in a given region are included in the multi-marker disease model, so that $P_1 = p(S \geq 1 | \mathcal{D})$. These posterior statistics are easily calculated from the PIPs using the variational approximation (see Supplementary Methods).

Since no pair of pathways stands out in Table 2 as having greater support than any other pair, we compute the posterior statistics $P_1$ by averaging over the different models with two enriched pathways, including models with BFs too small to be included in the table, weighting these models by their BFs. Implicitly, this assumes that all models with two enriched pathways are equally plausible a priori. The ability to average across models in this way is an advantage of adopting the Bayesian approach to model comparison, because it allows us to assess genetic associations in light of the enrichment evidence without having to choose a single pair of pathways. Suppose we have $m$ enrichment models $a^{(1)}, \ldots, a^{(m)}$ with corresponding Bayes factors $BF(a^{(1)}), \ldots, BF(a^{(m)})$, then $P_1$ for a given region is

\[
P_1 = \frac{\sum_{i=1}^{m} p(S \geq 1 | \mathcal{D}, a^{(i)}) BF(a^{(i)})}{\sum_{i=1}^{m} BF(a^{(i)})}. \tag{9}
\]

Further details about computation of posterior statistics are given in Supplementary Methods.

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for ways, we first get the pathway names and IDs by searching .gmt

From the Pathway Commons website, we download the BioCarta data because it was used in an previous pathway.

We retrieve most of the pathways from the Pathway Commons gene sets, whenever there is disagreement we include both gene sets, and assess evidence:

This can lead to large discrepancies in pathway gene sets, notably in the IL23-mediated signaling pathway. These two repositories include pathways from the same databases, but due to differences in versions of the databases and data processing procedure, there are discrepancies among pathways. At present, BioSystems ignores nesting relationships between pathways in the PID.

This lead to large discrepancies in pathway gene sets, notably in the IL23-mediated signaling pathway. Since we cannot fully account for all discrepancies in the BioSystems and Pathway Commons gene sets, whenever there is disagreement we include both gene sets, and assess evidence for enrichment of these gene sets separately in our analysis.

We download a version of the BioCarta database at www.openbioinformatics/gengen

We use this version of the BioCarta data because it was used in an previous pathway analysis of Crohn’s disease. We download version 3.01 of the PANTHER “sequence association” file from their FTP site. From the sequence association file, we retain lines containing ENSG* accession numbers (corresponding to human genes) and remove entries that do not map to Entrez gene IDs.

The numbers given in Table 4 are tabulated after discounting 213 pathways with less than two genes that map to the reference genome, and after removing 44 PID pathways from Pathway Commons that contain over 500 genes because their definitions include a large number of nested pathways. We include all groups of pathways except for two unusually large gene sets from the KEGG database that are unions of related pathways, “metabolic pathways” and “pathways in cancer.”

Appendix A: Supplementary Methods

Pathways

We retrieve most of the pathways from the Pathway Commons and NCBI BioSystems repositories. From the Pathway Commons website, we download the October 26, 2011 version of Gene Matrix Transposed (.gmt) file for homo sapiens. To retrieve BioSystems pathways, we first get the pathway names and IDs by searching ‘‘homo sapiens’’ [organism], then save the search result as a CSV file. Next, we download the November 15, 2011 version of the biosystems_gene file from the NCBI FTP site, which provides associations between genes and pathways. These two repositories include pathways from the same databases, but due to differences in versions of the databases and data processing procedure, there are discrepancies among pathways. At present, BioSystems ignores nesting relationships between pathways in the PID.

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Computation

The main difficulty in computing the Bayes factor (eq. 3) is the combinatorially large number of ways we can include 1

SNPs in the additive model of disease risk. In previous work 34, we described an approximation that yields an efficient procedure for computing the likelihood and PIPs. (Actually, this approximation was derived for the specific case when all variables have the same prior inclusion probability, or when $\theta = 0$, but it is straightforward to extend this approximation to the more general case with $\theta > 0$.) Once we have a recipe for efficiently computing $p(y | X, a, \theta, \theta)$, we are left with the task of computing a one-dimensional integral in the denominator of $Q$, and a double integral in the numerator. Each of these integrals is then approximated using simple numerical integration techniques.

The basic idea behind this approximation is to formulate a lower bound to the likelihood,

$p(y | X, a, \theta, \theta) \geq e^{F(\theta, \theta, \theta)}$, \hspace{1cm} (10)

then to adjust the free parameters, denoted by $\phi$, so that this bound is as tight as possible. (The exact form of $F(\theta, \theta, \theta, \phi)$ is derived in 35 and is given below.) This lower bound is formulated by introducing a probability distribution $q(\beta; \phi)$ that approximates the posterior of $\beta$ given $\theta_0$ and $\theta$ so that maximizing the lower bound corresponds to finding the approximating distribution that best matches the posterior. More precisely, it amounts to searching for the free parameters $\phi$ that minimize the Kullback-Leibler divergence between $q(\beta; \phi)$ and the posterior of $\beta$ given $\theta_0$ and $\theta$ 126. The trick to making this approach tractable lies in forcing $q(\beta; \phi)$ to observe a simple conditional independence property, as originally suggested by 10: each regression coefficient $\beta_j$ is independent of the other coefficients $a posteriori$ given $\theta_0$ and $\theta$. In other words, we restrict this distribution to be of the form

$q(\beta; \phi) = \prod_{j=1}^{p} q(\beta_j; \phi_j)$, \hspace{1cm} (11)

where $\phi_j$ is the set of free parameters corresponding to the $j$th factor.

For most SNPs, this conditional independence assumption is appropriate—most SNPs are unlinked because they are on separate chromosomes, or they are weakly linked because of recombination. In this case, the fully-factorized approximation $q(\beta; \phi)$ will closely recover the correct posterior distribution of the additive effects for these SNPs. But the conditional independence assumption is violated for SNPs in linkage disequilibrium. In that case, we do not expect to obtain accurate posterior statistics and, in practice, we find that the lower bound 110 can be a poor substitute to the correct likelihood. However, we are interested in accurate computation of BFs, not individual likelihoods, so what matters is whether $e^{F(\theta, \theta, \theta, \phi)}$ correctly captures the shape of the likelihood, or how the likelihood $p(y | X, a, \theta, \theta)$ changes as a function of $\theta_0$ and $\theta$; if the lower bound undershoots the exact likelihood by a constant factor across different settings of $\theta_0$ and $\theta$, this constant factor will cancel out in the BF. In 35, we show that the variational approximation (when the phenotype is a quantitative trait) can closely reproduce the shape of the likelihood, and can give accurate estimates of some posterior quantities, even when the conditional independence assumptions are not particularly appropriate. We caution, however, that the accuracy the approximation has only been assessed empirically, and we have no theoretical guarantees of its accuracy.
Computing the Bayes factor for one pathway

To start, we formulate a simple piecewise numerical approximation to the integrals in (10) based on Simpson’s rule (127). We replace each instance of the likelihood \( p(y | X, a, \theta_0, \theta) \) with its corresponding lower bound (10). Following the discussion above, we have

\[
BF(a) \approx \frac{I_{\text{null}}}{I_{\text{alt}}} = \frac{\sum_i \sum_j w_{ij} e^{F(\hat{\phi}, \theta_0, \phi(\theta_0), \phi(\hat{\theta}))} p(\theta(\hat{\theta})) p(\theta(j))}{\sum_i w_i e^{F(\hat{\phi}, \theta_0, \phi(\hat{\theta}), \theta(\hat{\theta}))} p(\theta(\hat{\theta}))},
\]

so the numerical approximation to the BF is

\[
BF(a) \approx \frac{I_{\text{null}}}{I_{\text{alt}}} = \frac{\sum_i \sum_{\theta_0} w_{i0} e^{F(\hat{\phi}, \theta_0, \phi(\theta_0), \phi(\hat{\theta}))} p(\theta(\hat{\theta}))}{\sum_i w_i e^{F(\hat{\phi}, \theta_0, \phi(\theta_0), \phi(\hat{\theta}))}},
\]

where \( w_i \) and \( w_{ij} \) are weights obtained by applying Simpson’s rule. To calculate the numerical approximation to the null likelihood, \( I_{\text{null}} \), we evaluate the lower bound at equally spaced points over the interval \([-6, -2]\). The likelihood under the alternative is a double integral, so we evaluate the lower bound at points on a regular grid over the rectangular region \( \theta_0 \in [-6, -2], \theta \in [0, 3] \). In eq. (13), the free parameters \( \phi \) are expressed as a function of \( \theta_0 \) and \( \theta \) because we adjust them separately for each setting of the hyperparameters \((\theta_0, \theta)\). (Optimizing \( \phi \) involves iterating coordinate ascent steps until these steps converge to stationary point which constitutes a locally optimal bound to the likelihood. Full details about the procedure to solve for \( \phi \) are given in [36]. This procedure scales linearly with the number of samples and the number of SNPs.) Adjusting the free parameters for each setting of the hyperparameters can be an costly endeavor for a large problem, so to reduce the expense of computing the BF we formulate piecewise numerical approximations to the integrals using a small number of equally spaced points at intervals of length 0.25, so \( \theta_0^{\text{(i)}} = \{ -6, -5.75, \ldots, -2 \} \) and \( \theta^{\text{(i)}} = \{ 0, 0.25, \ldots, 3 \} \). This coarse partitioning risks some loss of accuracy, especially if the posterior distribution of the hyperparameters is sharply peaked inside the subintervals, and a finer grid is certainly possible. An adaptive method that refines the subintervals in the piecewise approximation could have been used instead (127), but we stick to this simple scheme with equally spaced points at larger subintervals because it allows us to compute BFs for all \( \sim 3000 \) candidate pathways in a reasonable amount of time.

The analytic expression for the lower bound to the log-likelihood is derived in the Appendix of [36] and, for con-venience, we reproduce it here:

\[
F(\hat{\phi}, \theta_0, \theta, \phi) = \log \hat{\sigma}_0 + \frac{y^2}{2\hat{\nu}} + \sum_{i=1}^n \log \psi(\eta_i) + \frac{\nu(1 + \nu)}{2\hat{\nu}} y^T X r + \frac{\nu^2}{4\hat{\nu}} X^T U X r - \frac{1}{2} \sum_{j=1}^p \left( X^T U X \right)_{jj} \text{Var}[\beta_j] + \frac{\nu}{2} \sum_{j=1}^p \alpha_j \left[ 1 + \log \left( \frac{\sigma_j^2}{\sigma^2_0} \right) \right] - \frac{\nu}{2} \sum_{j=1}^p \alpha_j \log \left( \frac{1 - \alpha_j}{1 - \pi_j} \right) \approx \frac{I_{\text{null}}}{I_{\text{alt}}}.
\]

where the sigmoid function \( \psi(\eta) \) is defined above, and the prior inclusion probability \( \pi_j \) for each SNP is given by [2].

For this expression we introduce the following definitions: \( \alpha_j \) is the PIP for SNP \( j \) with respect to the approximating distribution \( \beta_j(\phi, \mu_2) \) and \( s_j^2 \) are the approximate mean and variance of coefficient \( \beta_j \) conditioned on being included in the model, \( \text{Var}[\beta_j] = \alpha_j (\mu_2^2 + s_j^2) - (\alpha_j \mu_2)^2 \) is the variance of \( \beta_j \) with respect to the approximating distribution, \( r \) is a column vector with entries \( r_j = \alpha_j \mu_2, \sigma_0 = 1/\sqrt{n} \) is the standard deviation of the intercept \( \beta_0 \) given \( \beta, \beta_0 = \hat{y}/\hat{u} \) is the posterior mode of the intercept \( \beta_0 \) when \( \beta = 0, (X^T U X)_{jj} \) is the \( j \)th diagonal entry of matrix product \( X^T U X \), and we define \( \hat{u} = \sum_{i=1}^n u_i, \hat{y} = \sum_{i=1}^n (y_i - \hat{y}), \hat{g} = \hat{y} - \frac{1}{2} \hat{\beta}_0 u, \hat{U} = U - uu^T/\hat{u} \) is a column vector with entries \( u_i = (\psi(\eta_i) - \frac{1}{2})/\eta_i \), and \( U \) is the \( n \times n \) matrix with diagonal entries \( u_i \).

In [36], to derive this analytic expression for the lower bound we made an additional approximation to the nonlinear factors appearing in the logistic regression likelihood \( p(y | X, \beta_0, \beta) \), following [128, 129]. This approximation introduces an additional set of free parameters, \( \eta = \{ \eta_1, \ldots, \eta_n \} \), so implicitly the lower bound (14) is a function of \( \eta \) as well. Like \( \phi \), we adjust \( \eta \) separately for each hyperparameter setting \((\theta_0, \theta)\). The procedure to solve for \( \eta \) is given in [36].

Since the coordinate ascent updates used to solve for \( \phi \) and \( \eta \) are only guaranteed to converge to a local maximum of the lower bound, the choice of starting point can affect the tightness of the lower bound, and the quality of the approximation. As we explain in [36], this issue can be addressed somewhat by using a common initialization \((\phi^{\text{(init)}}, \eta^{\text{(init)}})\) for the coordinate ascent updates across all grid points \((\theta_0^{\text{(i)}}, \theta^{\text{(i)}})\), in which this initialization is selected by first running the coordinate ascent procedure separately for each grid point, with random initializations for \( \phi \) and \( \eta \), then assigning \((\phi^{\text{(init)}}, \eta^{\text{(init)}})\) to the solution from the hyperparameter setting with the largest marginal likelihood estimate. In practice, when we follow this procedure we find that final estimates of BFs and posterior statistics vary only slightly when the analysis is re-run with different random initializations. However, we cannot guarantee the possibility that a new random starting point leads to the discovery of a much better approximation.
Computing the posterior inclusion probabilities and other posterior statistics

In this section, we describe computation of PIPs and other posterior statistics when a pathway, or a combination of pathways, is enriched. Computation of these quantities under the null hypothesis proceeds in a similar manner by setting \( \theta = 0 \).

Following the procedure for computing the BFs, we formulate a piecewise numerical approximation to the integral in (8), substituting each PIP conditioned on \( \theta_0 \) and \( \theta \) with the corresponding variational approximation, \( \alpha_k(\theta_0, \theta) \approx p(\beta_k \neq 0 \mid \mathcal{D}, \theta_0, \theta) \). This yields the following approximate PIP:

\[
\text{PIP}(k) \approx \sum_i \sum_j \tilde{w}_{ij} \alpha_k(\theta_0^{(i)}, \theta^{(j)}),
\]

where we define

\[
\tilde{w}_{ij} \propto p(\beta_1 = 0, \ldots, \beta_m = 0 \mid \mathcal{D}, \theta_0^{(i)}, \theta^{(j)}),
\]

such that \( \sum_i \sum_j \tilde{w}_{ij} = 1 \). Other posterior quantities are computed by averaging over \( \theta_0 \) and \( \theta \) in a similar way. For example, the posterior mean enrichment statistic is

\[
\bar{\theta} = E[\theta \mid \mathcal{D}]
\]

\[
= \int \theta \, p(\theta_0, \theta \mid \mathcal{D}) \, d\theta_0 \, d\theta \approx \sum_i \sum_j \tilde{w}_{ij} \, \theta^{(j)}.
\]

To compute credible intervals for \( \theta \), we add up the normalized weights \( \tilde{w}_{ij} \) over successively wider intervals of \( \theta \), beginning at the posterior mean \( \bar{\theta} \), until the sum of the normalized weights \( \tilde{w}_{ij} \) reaches 0.95. As a result, the credible intervals are at the same resolution as the grid points used for the numerical approximation.

The estimate of the posterior probability that at least one SNP in a given segment of the genome is included in the additive model of disease risk is

\[
P_1 \approx \sum_i \sum_j \tilde{w}_{ij} \, p(S \geq 1 \mid \mathcal{D}, \theta_0^{(i)}, \theta^{(j)})
\]

\[
= \sum_i \sum_j \tilde{w}_{ij} \left[ 1 - p(S = 0 \mid \mathcal{D}, \theta_0^{(i)}, \theta^{(j)}) \right].
\]

(17)

where \( S = n \) represents the event that \( n \) SNPs in the segment are included in the model. Assume without loss of generality that SNPs in the segment are labeled 1 through \( m \). Since the regression coefficients are independent under the fully-factorized approximating distribution given \( \theta_0 \) and \( \theta \), we have

\[
p(S = 0 \mid \mathcal{D}, \theta_0, \theta) = p(\beta_1 = 0 \land \cdots \land \beta_m = 0 \mid \mathcal{D}, \theta_0, \theta)
\]

\[
\approx \prod_{k=1}^m (1 - \alpha_k(\theta_0, \theta)),
\]

(18)

so our final estimate of this posterior statistic is

\[
P_1 \approx \sum_i \sum_j \tilde{w}_{ij} \left[ 1 - \prod_{k=1}^m (1 - \alpha_k(\theta_0^{(i)}, \theta^{(j)})) \right].
\]

(19)

To compute \( P_2 = p(S \geq 2 \mid \mathcal{D}) \) for a given segment, we observe that \( p(S = 2) = 1 - p(S = 1) = p(S = 0) \), and under the fully-factorized variational approximation, we have that

\[
p(S = 1 \mid \mathcal{D}, \theta_0, \theta)
\]

\[
= p(\beta_1 \neq 0 \land \beta_2 = 0 \land \cdots \land \beta_m = 0 \mid \mathcal{D}, \theta_0, \theta) + \cdots
\]

\[
+ p(\beta_1 = 0 \land \cdots \land \beta_{m-1} = 0 \land \beta_m \neq 0 \mid \mathcal{D}, \theta_0, \theta)
\]

\[
\approx \prod_{k=1}^m (1 - \alpha_k) \times \prod_{k=1}^m \frac{\alpha_k}{1 - \alpha_k}.
\]

(20)

Scaling computation to many pathways

Numerical integration together with the variational approximation makes it practical to compute BF(a) for one pathway, but computing BFs for thousands of pathways is still a costly undertaking. We introduce a simplifying assumption which, we show, yields substantial savings in computation. We make the assumption that SNPs outside the enriched pathway are unaffected by the pathway enrichment a posteriori. Formally, this means that \( p(\beta_j \mid \mathcal{D}, \theta_0, \theta) = p(\beta_j \mid \mathcal{D}, \theta_0, \theta = 0) \), where \( A \) is the set of SNPs assigned to the enriched pathway, and \( \bar{A} \) is the remaining set of SNPs. In other words, the posterior distribution of the regression coefficients for SNPs outside the enriched pathway remains the same under the null and enrichment models. With this assumption, the posterior distribution of \( \beta \) given the hyperparameters \( \theta_0 \) and \( \theta \) becomes

\[
p(\beta \mid \mathcal{D}, \theta_0, \theta) = p(\beta_A \mid \mathcal{D}, \theta_0, \theta, \beta_A) \times p(\beta_{\bar{A}} \mid \mathcal{D}, \theta_0, \theta = 0).
\]

(21)

This assumption amounts to conditioning on the additive effects of SNPs outside the enriched pathway.

It is of course possible that SNPs contributing evidence for pathway enrichment are correlated with SNPs outside the pathway, invalidating this assumption. But because we assign SNPs to pathways in contiguous blocks (we annotate all SNPs within 100 kb of a gene in the pathway; see Methods), and because the way we assign SNPs to genes is not precise (many SNPs assigned to a gene are probably not relevant to the gene), errors as a result of this assumption are expected to be minor compared to the imprecision in the SNP-pathway assignments. On the other hand, if we were to assign SNPs to pathways more precisely (e.g. a SNP known to modulate expression of a gene in the pathway), then this assumption would not be appropriate.

Next, we show how this assumption allows us to reuse computations. This assumption implies that, for any SNP \( j \) that is not assigned to the enriched pathway, \( q(\beta_j; \phi_j) \) remains the same under the null and enrichment models; that is, for any \( j \notin A \), \( \phi_j = \phi_j^* \), in which \( q(\beta; \phi) \) approximates the posterior distribution of \( \beta \) given \( \theta_0 \) and \( \theta = 0 \), and \( q(\beta; \phi^*) \) approximates the posterior distribution of \( \beta \) given \( \theta > 0 \). From this result, the lower bound can be written as

\[
F(\mathcal{D}, \theta_0, \theta, \phi^*) = F(\mathcal{D}, \theta_0, \theta = 0, \phi)
\]

\[
+ F(\{X_A, \hat{y}_A, \alpha_A = 1\}, \theta_0, \theta, \phi_A)
\]

\[
- F(\{X_A, \hat{y}_A, \alpha_A = 1\}, \theta_0, \theta = 0, \phi_A^*),
\]

(22)

where \( X_A \) is the matrix of genotypes for SNPs assigned to the enriched pathway, \( \phi_j^* \) is the set of free parameters corresponding to SNPs \( j \in A \), \( \alpha_A \) is the set of pathway annotations restricted to SNPs \( j \in A \) (so \( \alpha_A \) is a vector of ones), and \( \hat{y}_A = y - \hat{U}_A^T r_J \) is the vector of binary labels “corrected” for SNPs outside the pathway, where \( \hat{X}_A \) is the genotype matrix for SNPs \( j \notin A \), and \( r_J \) is the vector of entries \( r_J = \alpha_j \mu_j \) restricted to SNPs \( j \notin A \). Note that \( r_J \) is valid only if \( \eta \) is held constant.

Identity \( \underline{\text{22}} \) suggests a way to reuse our computations: once we have solved for \( q(\theta_0; \theta = 0) \), the free parameters \( \phi_0 \) that (locally) maximize the lower bound under the null hypothesis (\( \theta = 0 \)) for a given \( \theta_0 \), to solve for \( q(\theta_0; \theta = 0) \) for any \( \theta > 0 \), we only need to adjust the free parameters \( \phi_j \) corresponding to SNPs \( j \) in the enriched pathway. Crucially,
η must be held constant in (22), so for any θ > 0, we set η(θ,0,θ), the free parameters η adjusted for setting (θ0, θ) of the hyperparameters, to η(θ0, θ = 0).

Appendix B: Supplementary Results

Pathway database statistics

We observe a wide range in the number of genes assigned to each pathway (Fig. A.1A). Some of the larger gene sets are groups of related pathways in the Reactome and PID hierarchies. Out of ~23,000 genes in the reference genome, 9054 (~39%) are assigned to at least one pathway. The number of pathway assignments per gene varies widely (Fig. A.1B). Among genes assigned to at least one pathway, 95% are within 100 kb of a SNP, and 45% of SNPs are mapped to at least one gene in a pathway (Fig. A.1C). Figure A.1 depicts the hierarchical relationships among pathways in Table I.

More details on associations given enrichment of cytokine signaling genes

Here we supply a few more details about regions of the genome that show strong support for association only after accounting for enrichment of cytokine signaling: the MHC class II region, the IBDS locus, and a region at 17q21 near gene STAT3. Without pathway enrichment, the segment with the highest P1 at 17q21 is only 0.12, but this increases to 0.79 in the model in which cytokine signaling is enriched. This association was first identified in a meta-analysis [1], and was later confirmed in [2]. STAT3, the most compelling disease-susceptibility gene at this locus, plays a key role in Th17 cell differentiation and IL-23 signaling [76, 154, 155]. It has also been identified as a risk factor for other autoimmune diseases, including ulcerative colitis [94, 95].

Enrichment of cytokine signaling also yields greater support for an association in the IBDS region at position 5q31, increasing the probability of an included SNP from P1 = 0.18 to P1 = 0.81. The IBDS locus was first identified in a genome-wide study of individuals from Quebec [53], and this finding has since been replicated and refined by several studies [6, 7, 90, 132]. It was identified as a modest association for Crohn’s disease in the original report (Table 4 in [51]) with trend p-value 5.4 × 10−6 and BF = 104.54. The IBDS locus includes several candidate genes in a region of extensive linkage disequilibrium. Despite this, identification of this locus has suggested the contribution of gene variants affecting epithelial barrier integrity in Crohn’s disease pathogenesis [10, 132].

The MHC class II region, previously identified as a region showing moderate evidence of association [51], becomes a stronger association when cytokine signaling is enriched, as P1 increases from 0.49 to 0.98. The MHC is one of the most thoroughly studied regions of the human genome for its contribution to regulation of the immune system. Association of this locus (also known as IBD3) is widely replicated for Crohn’s disease [2, 57, 58, 61], ulcerative colitis [131] and, not surprisingly, many other autoimmune diseases [54, 133]. The extensive linkage disequilibrium across this gene-dense region complicates identification of disease-susceptibility variants; see [57] for a detailed examination of specific MHC genes contributing to Crohn’s disease risk. We also note that evidence for association of the MHC class II genes, including TNF, is low under the null in our analysis, with P1 = 0.08, and increases to P1 = 0.52 once we account for enrichment of cytokine signaling.

Additional results of sensitivity analysis

Under the null hypothesis, there is a clear trend in the overall effect of the choice of σ0 on the distribution of associations; we observe that the posterior mean of the genome-wide log-odds θ0 increases as σ0 decreases (Fig. B.2). For instance, the normal prior with standard deviation 0.06 corresponds to a posterior mean of θ0 = −3.4 and prior inclusion probabilities of roughly 10−3−4 ≈ 0.00044 under the null hypothesis, which is more than double the proportion of SNPs that are independent associations (0.00018) when σ0 = 0.1.

Fig. B.3 depicts the distribution of BF values for different choices of σ0. For most of the candidate pathways, the support for enrichment does not change much.
activated TAK1 mediates p38 MAPK activation

TAK1 activates NF-κB by phosphorylation and activation of IKKs complex

JNK phosphorylation and activation mediated by activated human TAK1

IL-3, IL-5 and GM-CSF signaling

Interleukin receptor SHC signaling

interferon gamma signaling

TCR signaling in naïve CD4+ T cells

IL12-mediated signaling events

IL27-mediated signaling events

CXCR4-mediated signaling events

Fig. B.1. Hierarchical relationships among Reactome and PID pathways listed in Table I and elsewhere in our analysis. For example, “signaling by interleukins” and “interferon signaling” (in addition to other pathways not shown) are part of “cytokine signaling in immune system.” Ellipses (· · ·) indicate that there are intermediate entries in the hierarchy not shown.

Fig. B.2. Posterior mean of $\theta_0$ for each choice of $\sigma_a$. Error bars depict 95% credible intervals.

Fig. B.3. Distribution of BF$s for different settings of $\sigma_a$. 