

Combining network-guided GWAS to discover susceptibility mechanisms to breast cancer

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Abstract

Systems biology provides a comprehensive approach to biomarker discovery and biological hypothesis building. In this work, we question the utility of six network methods to GWAS data, which search subnetworks highly associated to a phenotype. We interrogate a familial breast cancer GWAS focused on BRCA1/2 negative French women. By trading statistical astringency for biological meaningfulness, most network methods get more compelling results than standard SNP- and gene-level analyses, recovering causal subnetworks tightly related to cancer susceptibility. We perform an in-depth benchmarking of the methods with regards to size of the solution subnetwork, their utility as biomarkers, the stability of the solutions and their runtime. Interestingly, a combination solution subnetworks provided a concise subnetwork of 51 genes, enriched in known familial breast cancer susceptibility genes (FGFR2, TOX3, CASP8 and BLM) and more central than average. Additionally, it includes subnetworks of mechanisms related to cancer, like protein folding (HSPA1A, HSPA1B, and HSPA1L) or mitochondrial ribosomes (MRPS30, MRPS31, MRPS18B). We also observed a general disregulation in the neighborhood of COPS5, a gene related to multiple hallmarks of cancer.

Introduction

In human health, genome-wide association studies (GWAS) aim at quantifying how genetic variants predispose to complex diseases, like diabetes or some forms of cancer [1]. The most commonly studied variants are single-nucleotide polymorphisms (SNPs). Once the risk SNPs have been discovered, they can be used for diagnosis, guide the treatment, and deepen our understanding of the disease. To that end, in a typical retrospective study thousands of unrelated samples are genotyped: the cases, suffering the disease of

interest, and the controls from the general population. Then, a per-SNP statistical association test is conducted (e.g. χ^2). Those SNPs with a P-value lower than a conservative FWER threshold are candidates to further studies in an independent cohort.

GWAS successfully identifying thousands of variants underlying many common diseases [2]. However, the experimental setting also presents intrinsic challenges. Some of them stem from the high-dimensionality of the problem, as every GWAS to date study more variants than samples are genotyped. This limits the statistical power of the experiment, as only variants with large and moderate effects can be detected. However, most of the heritability can be explained by variants with small effects [3]. Additionally, to avoid false positives, a conservative multiple test correction is applied, typically Bonferroni. However, Bonferroni is known to be overly conservative when the statistical tests are correlated [4]. Another open issue is the interpretability of the results, as the functional consequences of most common variants are not well understood. The fact that SNPs in non-coding regions often top the results of GWAS portrays well this challenge. On top of that, recent large-sampled studies suggest that most of the genome contributes to a degree to any complex trait, in accordance with the infinitesimal model [5]. The omnigenic model [6] explain this by the dense interconnection between genes, influencing each others' behavior, which allows alterations in most genes to impact the "core" genes involved in a disease. A comprehensive statistical framework which includes the structure of biological data might address the aforementioned issues.

In this regard many authors turn to network biology to handle the complex interplay of biomolecules that lead to disease [7]. As its name suggests, network biology models biology as a network, where the biomolecules under study, often genes, are nodes, and selected functional relationships between them are the edges that link them. These functional relationships come from evidence that the genes jointly contribute to a biological function; for instance, their expression is correlated, or a protein-protein interaction between their gene products have been discovered. Under this view, complex diseases are not the consequence of a single altered gene, but of the interaction of multiple interdependent biomolecules [8]. In fact, an examination of biological networks shows that disease genes have differential properties [8] [9]. This was particularly true for cancer driver genes, which tended to be key players in connecting different densely-connected communities of genes. Additionally, as genes that contribute to a disease tend to participate in similar biological functions, guilt-by-association strategies have proved effective at identifying disease genes [10].

Network-based, biomarker discovery methods exploit the guilt-by-association strategy to identify disease genes on GWAS data. In essence, each SNP has a measure of association with the disease, given by the experiment, and functionally biological relationships, given by a network built on prior knowledge. Then, the problem becomes finding a functionally-related set of genes that is highly associated with the disease. Different solutions have been proposed to this problem, often stemming from divergent considerations of what the optimal solution set look like. Some methods strongly constrain the problem to certain kinds of subnetworks. Such is the extreme case of LEAN [11], which focuses on ‘star subnetworks’ i.e. instances where a gene and its direct interactors are associated with the disease. Other algorithms, like dmGWAS [12] and heinz [13], focus on interconnected genes with high association with the disease. However, they differ in their tolerance to include lowly associated nodes. Lastly, other methods explicitly consider the topology of the solution, favoring densely interconnected subnetworks; such is the case of HotNet2 [14], SConES [15], and SigMod [16].

In this work, we analyze the effectiveness of these six methods to discover new biomarkers on GWAS data. We focus on the GENESIS dataset [17], a homogeneous dataset of familial breast cancer in French population of European ancestry. After following a classical GWAS approach, we use these network-based methods to recover additional familial breast cancer biomarkers. Some of them are known, while others are specific to this dataset. Lastly, we carry out a comparison of the solutions obtained by the different methods, and aggregate them to obtain a consensus network of predisposition to familial breast cancer.

Methods

GENESIS

GENESIS is a GWAS study of familial breast cancer on the French population [17]. The index cases are patients with infiltrating mammary or ductal adenocarcinoma, who had sister with breast cancer, and negative for BRCA1 and BRCA2 mutations. The controls are unaffected colleagues and/or friends of the cases, born around the year of birth of the corresponding case (± 3 years). In total, the GENESIS dataset consists of 2577 samples, of which 1279 are controls and 1298 are cases. The genotyping platform was iCOGS, a custom Illumina array designed to study genetic susceptibility of hormone-related cancers [18]. It contains 210918 SNPs previously associated with cancer susceptibility, survival, or other cancer-related traits; and in selected

genes and pathways.

Preprocessing and quality control

We discarded SNPs with a minor allele frequency lower than 0.1%, not in Hardy - Weinberg equilibrium (P -value <0.001), and/or those with missing values on more than 10% of the samples. A subset of 20 duplicated SNPs in FGFR2 were also removed. In addition, we removed the samples with more than 10% missing genotypes, and an additional 28 samples with TODO. The final dataset included 1271 controls and 1280 cases, genotyped over 197083 SNPs.

We looked for population structure that could create confounding associations. A PCA revealed no differential population structure between cases and controls (Supplementary Figure 1). Independently, we did not find evidence of genomic inflation ($\lambda = 1.05$) either, further confirming the absence of confounding population structure.

High-score subnetwork search algorithms

SNP and gene association

To measure association between a genotype and the phenotype, we performed a per-SNP 1df χ^2 allelic test using PLINK v1.90 [19]. Then, we used VEGAS2v2 to compute the gene-level association score [20] from the SNP P -values. In order to map SNPs to genes we relied used their overlap on the sequence: all SNPs located within the boundaries of a gene, ± 50 kb, were mapped to that gene. To compute the gene association we used the 10% of SNPs with lowest P -values. We used the 62193 genes described in GENCODE 31 [21], although only 54612 could be mapped to at least one SNP. Out of those, we focused exclusively on the 32767 that could be mapped to an HGNC symbol. Out of the SNPs 197083 in iCOGS after quality control, 164037 mapped to at least one of these genes.

Gene-gene network

Out of the six methods tested, five use a gene-gene interaction network (Section Methods used), and their respective statistical frameworks are compatible with any type of network (protein interactions, gene coexpression, regulatory, etc.). However, in order to make the results comparable, we needed to apply all the methods to the same network. Hence, for practical reasons, we focused on a protein-protein interaction network (PPIN), as most of the

methods were designed to scale appropriately to it. We built our PPIN from both binary and co-complex interactions stored in the HINT database (release April 2019) [22]. Unless specified otherwise, we used only interactions coming from high-throughput experiments to avoid biasing the topology of the network by well-studied genes with more known interactions on average. Out of the 146722 interactions from high-throughput experiments that HINT stores, we were able to map 142541 to a pair of HGNC symbols. The scoring function for the nodes changed from method to method (Section Methods used).

Additionally, we compared the results of the aforementioned network with those obtained on a network built using interactions coming from both high-throughput and targeted studies. In that case, out of the 179332 interactions in HINT, we mapped 173797 to a pair of HGNC symbols.

SNP networks

SConES [15] is the only of the studied methods designed to handle SNP networks. As in gene networks, two SNPs are linked in a SNP network when there is evidence of shared functionality between two SNPs. The authors suggested three ways of building these networks: connecting the genotyped SNPs consecutive in the genomic sequence ("GS network"); interconnecting all the SNPs mapped to the same gene, on top of GS ("GM network"); and interconnecting all SNPs mapped to two genes for which a protein-protein interaction exists ("GI network"). Although we focused on the GI network, to make a fair comparison to the other methods, at different stages of the manuscript we also used GS and GM. For the GM network, we used the mapping described in Section SNP and gene association. For the GI network, we used the PPI as described in Section Gene-gene network. For all three networks the node score used is the association of the individual SNPs with the phenotype; specifically, we used the 1 d.f. χ^2 .

Mathematical notation

In this article, we refer to undirected, vertex-weighted networks, or graphs, $G = (V, E, w)$. $V = \{v_1, \dots, v_n\}$ refers to the vertices, with weights $w: V \rightarrow \mathbb{R}$. Equivalently, $E \subseteq \{\{x, y\} | x, y \in V \wedge x \neq y\}$ refers to the edges. When referring to a subnetwork S , V_S is the set of nodes in S and E_S is the set of edges in S . A special case of subgraphs are *connected* subgraphs, which occur when every node in the subgraph can be reached from any other node.

In addition, we use several matrices that describe different properties of

a graph. The described matrices are square, and have as many rows and columns as nodes are in the network. In fact, the element i,j represent a selected relationship between v_i and v_j . The adjacency matrix W_G contains a 1 when the corresponding nodes are connected through an edge, and 0 otherwise; the diagonal is zero. The degree matrix D_G is a diagonal matrix which contains the degree of the different nodes. Lastly, the Laplacian matrix L_G is defined as $L_G = D_G - W_G$.

Methods used

Table 1: Summary of the differences between the studied algorithms.

| Method | Field | Nodes | Exhaustive | Solution | Components | Input | Scoring |
|---------|-------|-------|------------|----------|------------|-----------|-----------------|
| heinz | Omics | Gene | Yes | - | 1 | Summary | BUM |
| HotNet2 | Omics | Gene | Yes | Modular | ≥ 1 | Summary | Local FDR |
| dmGWAS | GWAS | Gene | No | - | 1 | Summary | $-\log_{10}(P)$ |
| LEAN | Omics | Gene | Yes | Star | ≥ 1 | Summary | $-\log_{10}(P)$ |
| SConES | GWAS | SNP | Yes | Modular | ≥ 1 | Genotypes | χ^2 |
| SigMod | GWAS | Gene | Yes | Modular | 1 | Summary | $-\log_{10}(P)$ |

Beyond the assumption that genes that contribute to the same function will be nearby in the PPIN, they might be related to each other in diverse ways (densely interconnected modules, nodes around a hub, a path, etc.). That is not the only open problem: how to score the nodes, whether the affected mechanisms form a single connected component or several, how to frame the problem in a computationally efficient fashion, what is the best network to use, etc. In consequence, multiple solutions have been proposed. In this article, we examine six of them: five that explore the protein-protein interaction network, and one which explores SNP-networks. We selected methods that had an implementation that was programmatically accessible. Their main differences are summarized in Table 1.

heinz The goal of heinz is identifying the highest-scored connected subgraph on the network [13]. The authors propose a transformation of the genes' P-value into a score which is negative when no association with the phenotype is detected, and positive value when it is. To that end, the distribution of P-values is modeled by a beta-uniform model (BUM) parameterized by the desired FDR. In this situation the problem is NP-complete. To solve it efficiently it is re-casted as the Prize-Collecting

Steiner Tree Problem (PCST), which seeks to select the connected subnetwork S that maximizes the *profit* $p(S)$:

$$p(S) = \sum_{v \in V_S} p(v) - \sum_{e \in E_S} c(e).$$

where $p(v) = w(v) - w'$ is the *profit* of adding a node, $c(e) = w'$ is the *cost* of adding an edge, and $w' = \min_{v \in V_G} w(v)$. All three are positive quantities. PCST has an efficient, provable solution [23]. We used BioNet's implementation of heinz, available on Bioconductor [24, 25].

HotNet2 HotNet2 was developed to find connected subgraphs of genes frequently mutated in cancer [14]. To that end, it considers both the local topology of the network and the scores of the nodes. The former is captured by an insulated heat diffusion process: at the beginning, the score of the node determines its initial heat; iteratively each node yields heat to its "colder" neighbors, and receives heat from its "hotter" neighbors, while retaining part of its own (hence, *insulated*). This process continues until equilibrium is reached, and results in a similarity matrix F . F is used to compute the similarity matrix E that accounts also for similarities in node scores as

$$E = F \operatorname{diag}(w(V)),$$

where $\operatorname{diag}(w(V))$ is a diagonal matrix with the node scores in its diagonal. We scored the nodes as in [26], assigning a score of 0 for the genes with low probability of being associated to the disease, and $-\log_{10}(P\text{-value})$ to those likely to be. Both are separated using a local FDR approach [27]. To obtain densely connected subnetworks, HotNet2 prunes E , only preserving edges such that $w(E) > \delta$. Lastly, HotNet2 evaluates the statistical significance of the subnetworks by comparing their size to the size of networks obtained by permuting the node scores. HotNet2 has two parameters: the restart probability β , and the threshold heat δ . Both parameters are set automatically by the algorithm, and are robust [14]. HotNet2 is implemented in Python [28].

dmGWAS dmGWAS searches the connected subgraph with the highest local density in low P-values [12]. To that end it searches candidate subnetwork solutions using a greedy procedure involving the following steps:

1. Select a seed node.
2. Compute Stouffer's Z-score Z_m for the current subgraph as

$$Z_m = \frac{\sum z_i}{\sqrt{k}}$$

where k is the number of genes in the subgraph, $z_i = \phi^{-1}(1 - P\text{-value}_i)$, and ϕ^{-1} is the inverse normal distribution function.

3. Identify neighboring nodes i.e. nodes at distance $\leq d$. We set $d = 2$.
4. Add the neighboring nodes whose inclusion increases the Z_{m+1} more than $Z_m \times (1 + r)$. In our experiments, we set $r = 0.1$.
5. Repeat 2-4 until no increment $Z_m \times (1 + r)$ is possible.

Lastly, the module's Z-score is normalized as

$$Z_N = \frac{Z_m - \text{mean}(Z_m(\pi))}{\text{SD}(Z_m(\pi))}$$

where $Z_m(\pi)$ represent a vector with 100000 random subsets of the same number of genes.

We used the implementation of dmGWAS in the dmGWAS 3.0 R package [29]. We used the function *simpleChoose* to select the solution subnetwork, which aggregates the top 1% modules into the solution subnetwork.

LEAN LEAN searches deregulated "star" gene subnetworks i.e. subnetworks composed by one central node and all its interactors [11]. By imposing this restriction, LEAN is able to exhaustively test all such subnetworks (one per node). For a particular subnetwork of size m , the P-values corresponding to the involved nodes are ranked as $p_1 \leq \dots \leq p_m$. Then, k binomial tests are conducted, to compute the probability of having k out of m P-values lower or equal to p_k under the null hypothesis. The minimum of these k P-values is the score of the subnetwork. This score is transformed into a P-value through an empirical distribution obtained via a subsampling scheme, where sets of m genes are selected randomly, and their score computed. Lastly, P-values are corrected for multiple testing through a Benjamini-Hochberg correction. We used the implementation of LEAN from the LEANR R package [30].

SConES SConES searches the minimal, maximally interconnected, maximally associated subnetwork in a SNP graph [15]. Specifically, it solves the problem

$$\arg \max_{S \subseteq G} \underbrace{\sum_{v \in V_S} w(v)}_{\text{association}} - \lambda \underbrace{\sum_{v \in V_S} \sum_{u \notin V_S} W_{vu}}_{\text{connectivity}} - \underbrace{\eta |V_S|}_{\text{sparsity}} \quad (1)$$

where λ and η are hyperparameters that control the sparsity and the connectivity of the model. For two hyperparameters, the aforementioned problem has a unique solution, that SConES finds using a graph min-cut procedure. We used the version on SConES implemented in R package martini [31]. We selected λ and η by cross-validation, choosing the values that produce the most stable solution across folds. Note that the solution to the above problem can consist of several connected subnetworks which are disconnected from each other. In this case, the selected hyperparameters were $\eta = 3.51$, $\lambda = 210.29$ for SConES GS; $\eta = 3.51$, $\lambda = 97.61$ for SConES GM; and $\eta = 3.51$, $\lambda = 45.31$ for SConES GI.

SigMod SigMod aims at identifying the most densely connected gene subnetwork that is most strongly associated to the phenotype [16]. It addresses an optimization problem similar to that of SConES (Equation 1), but replacing the Laplacian matrix my the adjacency matrix (Section Mathematical notation).

$$\arg \max_{S \subseteq G} \underbrace{\sum_{v \in V_S} w(v)}_{\text{association}} + \lambda \underbrace{\sum_{v \in V_S} \sum_{u \in V_S} W_{vu}}_{\text{connectivity}} - \underbrace{\eta |V_S|}_{\text{sparsity}} .$$

As SConES, this optimization problem can also be solved by a graph min-cut approach.

SigMod presents two important additional differences with SConES. First it is designed for gene-gene networks. Second, it returns a single connected subnetwork, which it achieves by exploring a grid of hyperparameters and processing their respective solutions. Specifically, for the range of $\lambda = \lambda_{\min}, \dots, \lambda_{\max}$ for the same η , it prioritizes the solution with the largest change in size from λ_n to λ_{n+1} . Such a large change implies that the network is strongly interconnected. This

results in one candidate solution for each η , which are processed by removing any node not connected to any other. A score is assigned to each candidate solution by summing their node scores and normalizing by size. The candidate solution with the highest standardized score is the chosen solution. SigMod is implemented in an R package [32].

Mapping back and forth between gene methods and SConES

In this work dealt with multiple methods, which use GWAS data at different levels. VEGAS2 compute gene statistics from SNP statistics, which are then used by five gene-based network methods to find a subnetwork associated with familial breast cancer. In order to obtain a list of SNP biomarkers from these gene subnetworks, we consider all the SNPs that can be mapped to that gene as selected by the method. SConES is the opposite case: it performs selection on a network of SNPs. In this case, when analyzing the genes selected by SConES, we consider any gene that can be mapped to any of the selected SNPs as selected as well.

Consensus network

The different high-weight subnetwork discovery algorithms make different assumptions on the properties of the solutions, and employ different strategies to find them. Hence, combining the outcome of the different approaches might provide a more complete outlook on the specific alterations on the GENESIS dataset. We built such consensus network by retaining the nodes that were selected by at least two of the methods. We combined the results of 6 methods: heinz, HotNet2, dmGWAS, LEAN, SConES GI, and SigMod.

Validation of selected biomarkers

Classification accuracy of selected biomarkers

A desirable solution is one that is sparse, while offering a good predictor power on unseen samples. We evaluated the predicting power of the SNPs selected by the different methods through the performance of an L1-penalized logistic regression trained exclusively on those SNPs to predict the outcome (case/control). The L1 penalty helps to account for LD to reduce the size of the active set, while improving the generalization of the classifier. The value of the λ , which controls the size of the coefficients, was set by cross-validation. To that end, we used the different network-methods on a random subset of 80% of the samples. On this same subset we trained our classifier

exclusively on the SNPs selected by a particular method. When the method retrieved a list of genes (all of them except SConES), all the SNPs mapped to any of those genes were used. Then we evaluated performance of the classifier on the remaining 20% of the dataset. We repeated this procedure 5 times to estimate the average and the deviation of the different performance measures. The different performance measures we used where: size of the solution, size of the active set, specificity, sensitivity and average Pearson’s correlation between different runs. To obtain a baseline, we also performed the procedure using all the SNPs. Another desirable property is that the method retrieves a good candidate causal subnetwork. In consequence, we compared the outcome of each of the methods to the consensus subnetwork of all the solutions (Section Consensus network).

Biological relevance of the genes

An alternative way to validate the results is comparing our results to an external dataset. For that purpose, we recovered a list of 153 genes associated to familial breast cancer from DisGeNET [33]. Across this article we refer to these genes as *familial breast cancer genes*.

Additionally, we used the summary statistics from the Breast Cancer Association Consortium (BCAC) [34]. BCAC is one of the largest efforts in GWAS, with over 120000 women from European ancestry, albeit from different countries. As opposed to GENESIS, samples were not selected based on family history, and hence is enriched in sporadic breast cancers. Another difference is that BCAC is a relatively heterogeneous study on a pan-European sample, while GENESIS is a homogeneous dataset focused on the French population. Despite these differences, there should be shared genetic architecture. On top of that, that overlap should become more notorious when the results are aggregated at the gene level. For that purpose, we computed the gene association as in Section SNP and gene association. iCOGS array was used for genotyping in BCAC [18], the same array as for GENESIS [17]. Although imputed data is available, we used exclusively the SNPs available on GENESIS after quality control to make the results comparable.

Code availability

This work required developing computational pipelines for several GWAS analyses, such as physical mapping of SNPs, computing gene scores, and perform six different network-based analyses. For each of those processes, a streamlined, project-agnostic pipeline with a clear interface was created.

They are compiled in the following GitHub repository: <https://github.com/hclimente/gwas-tools>. The code that applies these pipelines to the GENESIS project, as well as the code that reproduces all the analyses in this article are available at <https://github.com/hclimente/genewa>. All the produced gene subnetworks were deposited on NDEX (<http://www.ndexbio.org>), under the UUID e9b0e22a-e9b0-11e9-bb65-0ac135e8bacf.

Results

FGFR2 is strongly associated with familial breast cancer

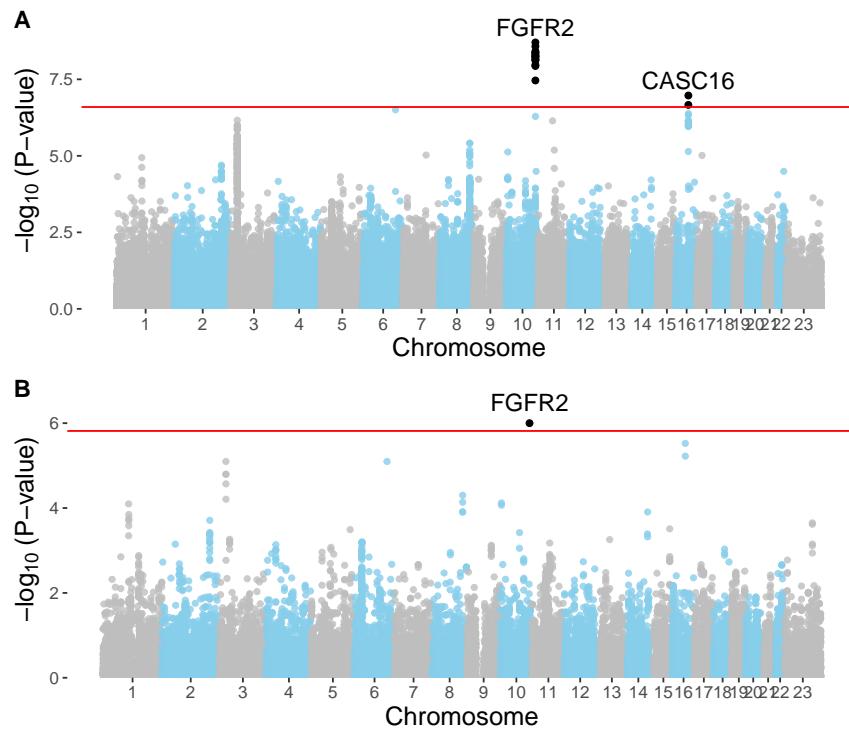


Figure 1: Association in GENESIS. The red line represents the Bonferroni threshold. **(A)** SNP association, measured from the outcome of a 1df χ^2 allelic test. SNPs that are within a coding gene, or within 50 kilobases of its boundaries, are annotated. The Bonferroni threshold is 2.54×10^{-7} . **(B)** Gene association, measured by P-value of VEGAS2v2 [20] using the 10% of SNPs with the lowest P-values. The Bonferroni threshold is 1.53×10^{-6} .

We conducted association analyses in the GENESIS dataset at both the SNP and the gene levels (Section SNP and gene association). Two genomic regions have a P-value lower than the Bonferroni threshold in chromosomes 10 and 16 (Figure 1A). The former overlaps with gene FGFR2; the latter with CASC16, and its located near the protein-coding gene TOX3. Variants in both FGFR2 and TOX3 were related to breast cancer susceptibility in other cohorts negative for BRCA1/2 [35]. Only the peak in chromosome 10 replicated in the gene-level analysis, with FGFR2 just above the threshold of significance (Figure 1B).

These results show the overlap in the genetic architecture of the disease between the French population and other cohorts, especially at the gene level. In addition, there are other regions highly associated with familial breast cancer, albeit well above the conventional threshold of significance. The most prominent regions, which have been associated to breast cancer susceptibility in the past, are 3p24 [36], and 8q24 [37]. This motivates exploring network methods, which trade statistical significance for biological relevance.

Network methods successfully identify genes linked to breast cancer

Table 2: Summary statistics on the results of multiple network methods on the gene-gene interaction network. The first row contains the summary statistics on the whole network.

| Network | # genes | # edges | betweenness | \hat{P}_{gene} | $\rho_{\text{consensus}}$ |
|-----------|---------|---------|-------------|-------------------------|---------------------------|
| HT HINT | 13619 | 142541 | 16706 | 0.46 | 0.066 |
| Consensus | 55 | 117 | 74062 | 0.0051 | 1 |
| dmGWAS | 194 | 450 | 49115 | 0.19 | 0.41 |
| heinz | 4 | 3 | 113633 | 0.0012 | 0.21 |
| HotNet2 | 440 | 374 | 7739 | 0.048 | 0.31 |
| LEAN | 0 | 0 | - | - | - |
| SConES GI | 0 | 0 | - | - | - |
| SigMod | 142 | 249 | 92603 | 0.0083 | 0.73 |

We applied six network methods to the GENESIS dataset (Section Methods used), obtaining six solutions (Supplementary files 1 and 2): one for each of the five gene-based methods (Section Gene-gene network), and one for SConES GI (Section SNP networks). The solutions are very heterogeneous (Table 2 and Supplementary table 1): none of the subnetworks examined by LEAN is significant (adjusted P-value < 0.05), while HotNet2 produced the

largest solution subnetwork with 440 genes. (SConES GI failed to recover genes in the PPIN, but it recovered genomic regions that can be mapped to other genes.) Four of the methods succeeded at recovering genes involved in the disease, as their subnetworks were enriched in familial breast cancer genes (dmGWAS, heinz, HotNet2, and SigMod, Fisher's exact test one-sided P-value < 0.03). We also compared the outcome of the network methods to the association tests conducted on the European cohort of the Breast Cancer Association Consortium (BCAC) [34] (Supplementary Figure 4). Encouragingly, every solution subnetwork is enriched in genes or SNPs that are Bonferroni-significant in BCAC. This confirms the capability of network methods to find the same signal as in more powered studies by leveraging on prior knowledge.

In fact, the solution subnetworks present other desirable properties. First, all solution subnetworks except LEAN's are, on average, more strongly associated to familial breast cancer than the whole HINT protein-protein interaction network. Although there are nuances: heinz strongly favored highly associated genes, while dmGWAS is less conservative (median gene P-value 0.0012 and 0.19, respectively). Also, the genes in three solution subnetworks display on average a higher betweenness centrality than the rest of the genes, a difference that is significant in three solutions (dmGWAS, and SigMod, Wilcoxon rank-sum test P-value < 1.4×10^{-21}). This agrees with the notion that disease genes are more central than other, non-essential genes [9]. We observe that this conclusion hold in this disease, as familial breast cancer genes have higher betweenness centrality than others (one-tailed Wilcoxon rank-sum test P-value = 2.64×10^{-5} , Supplementary figure 6C). Interestingly, SConES' selected SNPs are also more central than the average SNP (Supplementary table 1), suggesting that causal SNPs are also more central than unrelated SNPs.

As the solutions were quite different from each other it is hard to draw joint conclusions. The 4-gene solution selected by heinz includes the familial breast cancer gene TOX3, in region 16q12. By dealing with SNP networks, SConES studies the association of non-coding regions, as well as SNPs in any gene, coding or else. In fact, SConES GI, which adds to GM the interactions between genes, retrieves 4 subnetworks in intergenic regions, and 1 overlapping an RNA gene (RNU6-420P). SigMod, despite being related to SConES, produces a vastly different, large solution. On top of recovering three familial breast cancer genes, a keratin-based region of its subnetwork affects the cytoskeleton (*structural constituent of cytoskeleton*, GO enrichment's adjusted P-value = 9.10×10^{-4}), a potentially novel susceptibility mechanisms to cancer. Interestingly, dmGWAS solution is also related to

cytoskeleton (*tubulin binding*, GO enrichment's adjusted P-value = 0.031). But, additionally, it includes a submodule of proteins related to *unfolded protein binding* (GO enrichment's adjusted P-value = 0.045), which was related to cancer susceptibility [38]. Lastly, HotNet2 produced 135 subnetworks, 115 of which have less than five genes. The second largest subnetwork (13 nodes), contains two familial breast cancer genes: CASP8 and BLM.

heinz retrieves a small, highly informative set of biomarkers in a fast and stable fashion

As the methods produced such different results, we compared their solutions in a 5-fold subsampling setting (Section Classification accuracy of selected biomarkers). Specifically, we measured the following properties (Figure 2): (i) size of the solution subnetwork; (ii) stability; (iii) sensitivity and specificity of an L1-penalized logistic regression on the selected SNPs; and (iv) computational runtime.

Both solution size and active set of SNPs selected by Lasso varies greatly between the different methods (Figure 2A). heinz has the smallest solutions, with an average of 182 selected our of which 5.6% (10.2) are selected by Lasso. The largest solutions come from SConES GM (4548.6 SNPs), and dmGWAS (4307.4 SNPs). Interestingly, SigMod and SConES GI have the highest proportion of the selected SNPs that go into the active set (11.47 and 10.3% respectively). This suggests those methods are selecting more informative SNPs on average.

The sensitivity and specificity of the classifier on the testing data informs us about the usefulness of the selected SNPs as patient classification (Figure 2B). All classifiers' sensitivities were in the 0.38 - 0.69 range; the specificities, between 0.40 and 0.70. On average, SConES GS had the highest sensitivity (0.57); heinz, the highest specificity (0.56). Both SConES GS and SConES GM had on average better sensitivity than the classifier trained on all the SNPs, and dmGWAS and heinz superior specificities. However, the differences them were negligible, well within the 95% confidence interval.

The stability of a method measures its ability to select the same SNPs in face of perturbations on the data. We measured it by computing the pairwise Pearson's correlation between all pairs of solutions (Figure 2C). Heinz's displayed a high stability in our benchmark, consistently selecting the same SNPs over the 5 subsamples. LEAN also showed a high stability consistently selecting no SNP.

In terms of computational runtime, the fastest method was heinz (Figure 2D), which leverages on its ability to find efficiently the solution in a

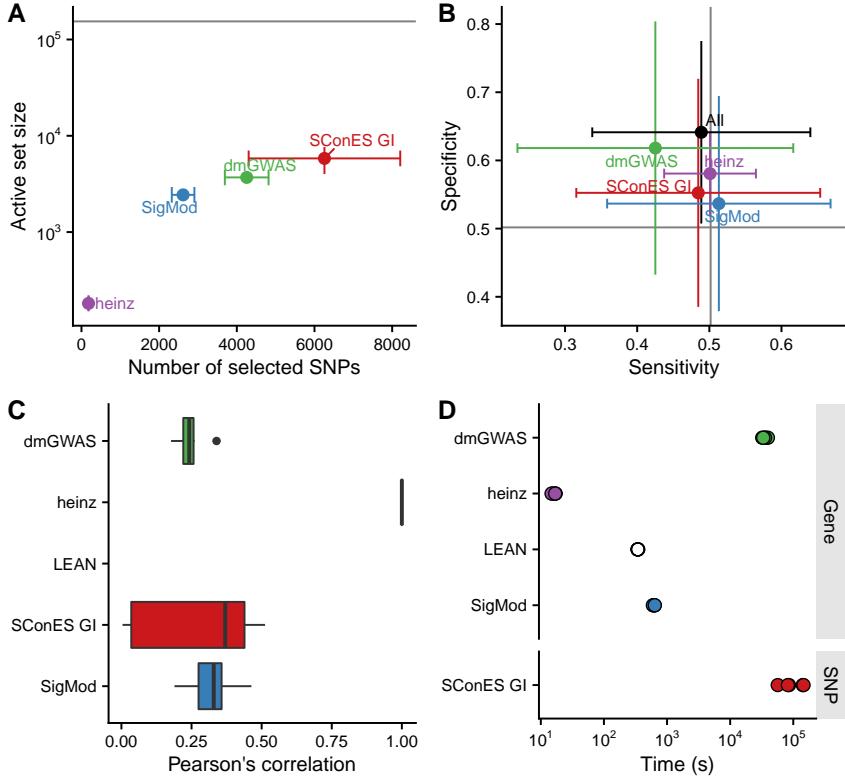


Figure 2: Comparison of network-based GWAS methods on GENESIS. Each method was run 5 times of a random subset of the samples, and tested on the remaining samples (Section Classification accuracy of selected biomarkers). **(A)** Number of SNPs selected by each method and number of SNPs on the active set used by the Lasso classifier. Points are the average over the 5 runs; lines represent the standard error of the mean. The horizontal grey line represents the average active set of Lasso using all the SNPs. **(B)** Sensitivity and specificity on testing set of the L1-penalized logistic regression trained on the features selected by each of the methods. In addition, the performance of the classifier trained on all SNPs is displayed. Points are the average over the 5 runs; lines represent the standard error of the mean. **(C)** Pairwise Pearson’s correlations of the solutions used by different methods. A Pearson’s correlation of 1 means the two solutions are the same. A Pearson’s correlation of 0 means that there is no SNP in common between the two solutions. **(D)** Runtime of the evaluated methods, by type of network used (gene or SNP). The gene network-based methods required an additional 119980 seconds (1 day and 9.33 hours) on average to compute the gene scores from SNP summary statistics (not included in the displayed Time).

few seconds. The slowest method was SConES using the GI network, with approximately 1 day and 2.38 hours on average. Including the time required to compute the gene scores, however, slows down considerably gene-based methods; on this benchmark, that step took on average 1 day and 9.33 hours. Considering that time, dmGWAS is the slowest method, taking 1 day and 21.81 hours on average.

No solution is perfect

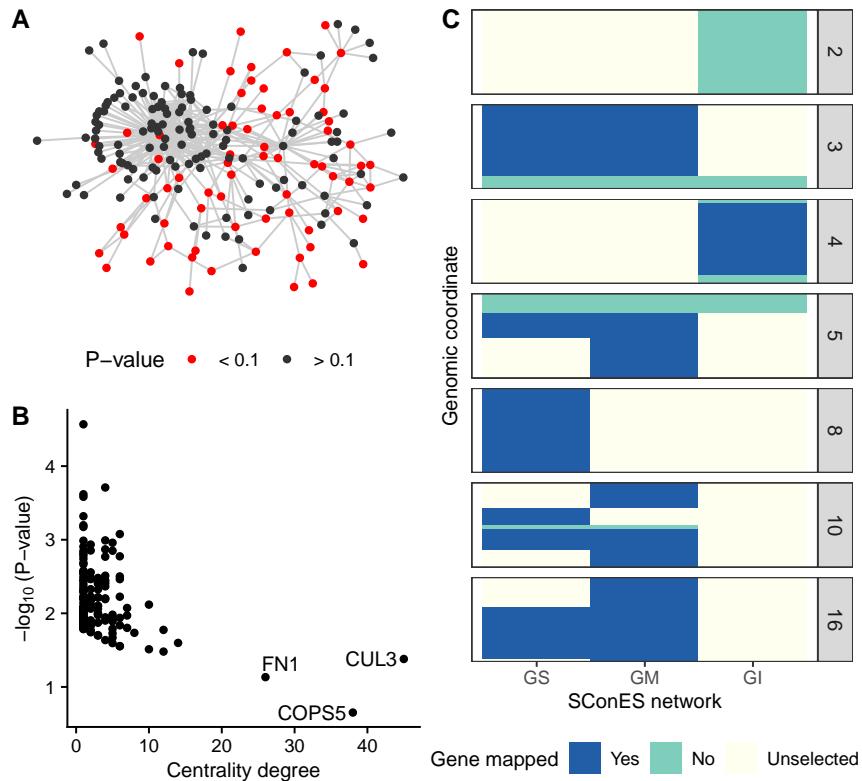


Figure 3: Drawbacks confronted when using network guided methods. **(A)** dmGWAS solution subnetwork. Genes with a P-value < 0.1 are highlighted in red. **(B)** Centrality degree and $-\log_{10}$ of the VEGAS P-value for the nodes in SigMod solution subnetwork. **(C)** Genomic regions where either SConES GS, GM or GI select SNPs.

In practice, and despite their similarities and their involvement in cancer mechanisms, the solutions are remarkably different (Supplementary figure

2A). That is due to the particulars of the methods, and directly or indirectly, they provide information about the dataset. For instance, the fact that LEAN did not provide any biomarkers implies that there is no gene such that both itself and its environment are on average strongly associated with the disease.

In this dataset, heinz's solution is very conservative, providing a small solution with the lowest median P-value for the subnetwork (Table 2). Due to this parsimonious and highly associated solution, it was the best method to select a set of good biomarkers for classification. (Figure 2B). Its conservativeness stems from its preprocessing step, which models the gene p-values as a mixture model of a beta and a uniform distribution, controlled by an FDR parameter. Due to the limited signal at the gene level in this dataset (Figure 1B), only 36 of them are considered to be associated to the disease. Hence, heinz's solution subnetwork consists only of 4 genes, which does not provide much insight of the biology of cancer. Importantly, it ignores genes that are strongly associated to cancer in this dataset like FGFR2.

On the other end of the spectrum, we have large solutions provided by dmGWAS, HotNet2, and SigMod. dmGWAS' subnetwork is the least associated subnetwork on average. This is due to the greedy framework it uses, which considers all nodes at distance 2 of the examined, and accepts weakly associated genes if they are linked to another, strongly associated one. This compounds when aggregating the results of successive greedy searches, leading to a large, tightly connected cluster of unassociated genes (Figure 3A). SigMod displays the same tendency, as the most central genes are the least associated to the disease (Figure 3B). This relatively low signal-to-noise ratio combined with the large solution requires additional analyses to draw conclusions, such as enrichment analyses. In the same line, HotNet2 subnetwork is even harder to interpret, being composed of 440 genes divided into 135 subnetworks. Lastly, SigMod misses some of the most strongly associated, familial breast cancer genes in the dataset, like FGFR2 and TOX3.

By virtue of using a SNP subnetwork, SConES analyzes each SNP in their context. Thanks to that, it selects SNPs in genes none of whose interactors are associated to the disease, as well as SNPs in non-coding regions or in non-interacting genes. In fact, due to linkage disequilibrium, such genes are favored by SConES, as selecting one favors selecting another one. This might explain why the GS and GM networks, heavily affected by linkage disequilibrium, produce similar results (Supplementary figure 2B). On the other hand, SConES penalizes selecting SNPs and not their neighbors. This makes it conservative regarding SNPs with many interactions, for instance those mapped to hubs in the PPIN. Influenced by this, SConES GI did not

select any protein coding gene, despite selecting similar regions as SConES GS (Figure 3C). In fact SConES GS and SConES GM select regions related to breast cancer, like 16q12 (TOX3, Section FGFR2 is strongly associated with familial breast cancer), 3p24 (SLC4A7/NEK10 [39]), 5p12 (FGF10, MRPS30 [40]), and 10q26 (FGFR2, Section FGFR2 is strongly associated with familial breast cancer). On top of that only SConES GS selects region 8q24 (POU5F1B [41]). We hypothesize that the lack of results on the PPIN network of SConES GI and LEAN is the same: the lack of joint association of a module. Although in the case of SConES other hyperparameters could lead to a more informative solution (lower λ), finding the appropriate configuration is hard. Also, the iCOGS platform is not a real GWAS experiment: the genome is not unbiasedly surveyed, some regions are fine-mapped - which might distort gene structure in GM and GI networks- while others are under studied - hurting the accuracy with which the GS network captures the genome structure.

Aggregating solutions provides insights into the biology of cancer

To leverage on the strengths of each of the methods and compensate their respective weaknesses, we built a consensus subnetwork that captures the mechanisms most shared among the solution subnetworks (Section Consensus network). The consensus subnetwork (Figure 4) contains 93 genes and is enriched in familial breast cancer genes (Fisher's exact test P-value = 7.8×10^{-5}). Due to the limited overlap between methods, only 20 genes were common to more than two of them (Supplementary figure 6A). Encouragingly, the more methods selected a gene, the higher its association was (Supplementary figure 6B). The consensus subnetwork is not completely connected: out of the 93 genes, the largest connected subnetwork includes only 49. A GO enrichment analysis showed that this component is related to two major cellular processes: unfolded protein binding (adjusted P-value = 0.02), and poly(U) RNA binding (adjusted P-value = 0.04). We found support in the literature of the involvement of each of these functions in the development of cancer. The consensus network also contains a protein directly involved in caspase-mediated apoptosis, CASP8 (VEGAS P-value = 1.95×10^{-4}). This is related to the enriched activity, *unfolded protein binding*, which inhibits caspase-dependent apoptosis, raising the chances of developing cancer [38]. It involves three Hsp70 chaperones of the consensus subnetwork: HSPA1A, HSPA1B, and HSPA1L. They all are closely encoded in 6p21. In fact out of the 22 SNPs that map to any of these three genes, 9 map to all of them,

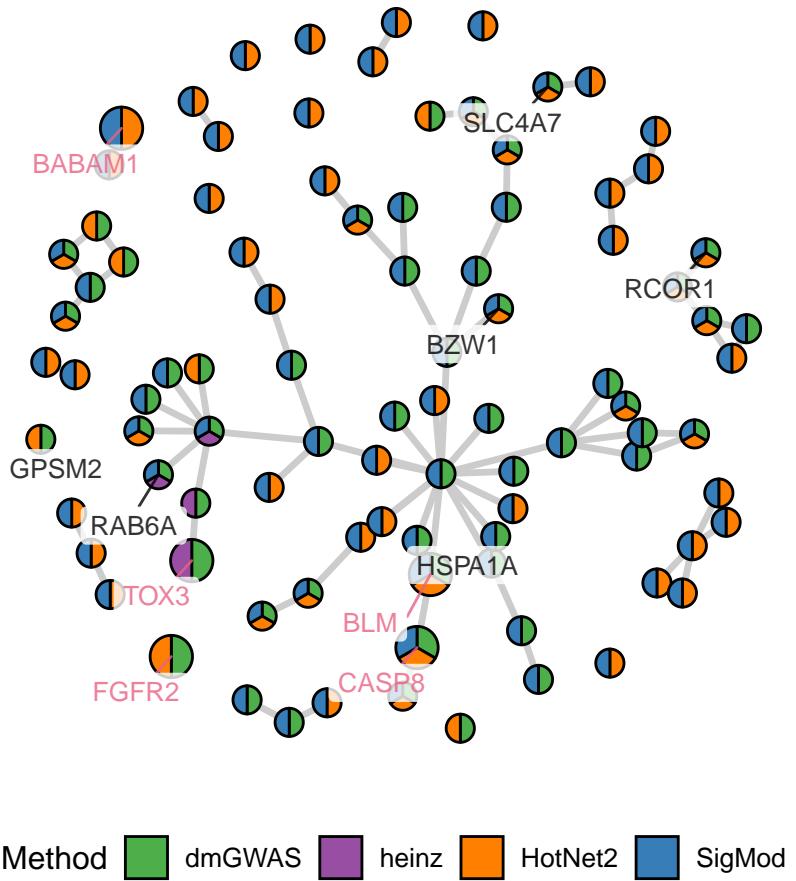


Figure 4: Consensus subnetwork on GENESIS (Section Consensus network). Each node is represented by a pie chart, which accounts the methods that selected it. The labeled genes have a VEGAS2 P-value < 0.001 and/or are known familial breast cancer genes (colored in pink).

and 4 to two, making hard to disentangle their association. HSPA1A was the most strongly associated one (VEGAS P-value = 8.37×10^{-4}).

We also examined the topological properties of the nodes. The genes in the consensus network have higher betweenness centrality than the rest of the genes (Wilcoxon rank-sum test P-value = 4.29×10^{-18}). Interestingly, within genes in the consensus network, cancer genes are as central as non-cancer genes (Wilcoxon rank-sum test P-value = 0.57). Centrality, however, is weakly anti-correlated with association to the disease (Pearson correlation coefficient = -0.26, Supplementary figure 6D), which suggests that some highly central genes were selected because they were on the shortest path between two highly associated genes. In view of this, we hypothesize that highly central genes might contribute to the heritability through consistent alterations of its neighborhood, consistent with the omnigenic model of disease [6]. For instance, the most central node in the consensus network is COPS5 (Supplementary figure 7), a gene related to multiple hallmarks of cancer and which is overexpressed in multiple tumors, including breast and ovarian cancer [42]. Despite its lack of association in GENESIS (VEGAS P-value = 0.22), its neighbors in the consensus subnetwork are consistently associated (median VEGAS P-value = 0.006).

Remarkably, 3 of the 51 genes are not linked to any other gene in the subnetwork, as they do not have a consistently altered neighborhood. One of them is the familial breast cancer gene FGFR2 (Section FGFR2 is strongly associated with familial breast cancer); the other two are SLC4A7 and MRPS30. SLC4A7 (VEGAS P-value = 2.70×10^5) is a gene encoding a sodium bicarbonate cotransporter, a function that was linked to cancer in the past [?]. In the past the genomic region containing both SLC4A7 and nearby gene NEK10 (VEGAS P-value = 1.56×10^{-5}) were associated with familial breast cancer [39]. NEK10 is a gene that might be involved in cell-cycle control, but it is absent from the PPIN and hence it could not be studied by gene methods. Despite that, the fact that both dmGWAS and SigMod find links between SLC4A7 and the rest of the subnetwork supports the notion that SLC4A7 is the responsible for cancer susceptibility. The other gene, MRPS30 (VEGAS P-value = 0.001), encodes a mitochondrial ribosomal protein and was also linked to breast cancer susceptibility [40]. Albeit disconnected from MRPS30, the consensus network includes a 2-node subnetwork composed of two mitochondrial ribosomal protein (MRPS31 - VEGAS P-value = 7.67×10^{-3} - and MRPS18B - VEGAS P-value = 7.92×10^{-3}), which suggests an involvement of mitochondrial ribosomes in carcinogenesis [?].

Hindrances of network analyses

The strength of network-based analyses comes from leveraging prior knowledge to boost discovery. In consequence, they falter in front of understudied genes, especially those not in the network. Out of the 32767 genes that we can map the genotyped SNPs, 60.7% (19887) are not in the protein-protein interaction network. The majority of those (14660) are non-coding genes, mainly lncRNA, miRNA, and snRNA (Supplementary figure 3). The importance of these genes, like CASC16, is highlighted in Section FGFR2 is strongly associated with familial breast cancer. Among the excluded protein-coding genes we find genes like NEK10 (P-value 1.6×10^{-5}), linked to breast cancer susceptibility [39], or POU5F1B, linked to prostate cancer [41]. However, on average protein coding genes absent from the PPIN are less associated with this phenotype (Wilcoxon rank-sum P-value = 2.79×10^{-8} , median P-values of 0.43 and 0.47). As we are dealing with high-throughput interactions, such difference cannot be due to well-known genes having more known interactions. As disease genes tend to be more central [9], we hypothesize that it is due to interactions between central genes being more likely. It is worth noting that network approaches that do not use PPIs, like SConES GS and GM, did recover SNPs in NEK10 and CASC16. Lastly, all the methods rely heavily on how SNPs are mapped to genes. In Section FGFR2 is strongly associated with familial breast cancer we highlight ambiguities that appear when genes overlap or are in linkage disequilibrium.

As not all databases compile the same interactions, the choice of the PPIN determines the final output. Specifically, in this work we used exclusively interactions from HINT from high-throughput experiments. This responds to concerns of some authors about biases introduced by adding interactions coming from targeted studies in the literature [43, 22]. It is a "rich getting richer" phenomenon, where popular genes have a higher proportion of their interactions described. On the other hand, one study found that the best predictor of the performance of a network for disease gene discovery is the size of the network [10]. This would support using the largest amount of interactions. To clarify their impact on this study, we compared the impact of using only physical interactions from high-throughput experiment versus interactions from both high-throughput and the literature (Section Gene-gene network). For most of the methods using a larger network did not greatly impact the size or the stability of the solution, the classification accuracy, or the runtime (Supplementary figure 5).

Discussion

In this article we evaluate the viability of systems biology approach to GWAS, and examine a GWAS dataset on familial breast cancer focused on BRCA1/2 negative French women. Systems biology addresses two of the largest GWAS issues: interpretability and an overly conservative statistical framework that hinders discovery. This is achieved by considering the biological context of each of the genes and SNPs, and selecting a threshold of association based on it. However, the method of choice is unclear. Based on divergent considerations of what the desired set of biomarkers is, several methods for network-guided biomarker discovery have been proposed. In this article we reviewed the performance of six network-guided of them on GWAS. Despite their differences, most of them produced a relevant subset of biomarkers, recovering known familial breast cancer genes. We also discuss the limitations of such analyses, related to the lack of known interactions around some genes.

The studied network methods differ in what the optimal solution subnetwork looks like. On the one hand, SConES and heinz prefer small highly associated solutions. On the other hand, SigMod and dmGWAS gravitate towards larger, less associated solutions which provide a wide overview of the biological context. While the former provide a reduced set of biomarkers, the latter deepen our understanding of the disease and provide biological hypothesis. They are not exempt of limitations. dmGWAS and SigMod's solution's size require further analyses, which risk oversimplifying its richness. Also, incautious practitioners might be misled by some genes, which are very central in the solution subnetworks, while being weakly associated. Nonetheless, they are pushed into the solution by their privileged topological properties. On the other end, conservative solutions, like SConES GI and heinz might not shed much light on the etiology of the disease.

To overcome the problems posed by the individual methods while exploiting their strengths, we propose combining them into a consensus subnetwork. We use a straightforward aggregation to generate it, including any node that was recovered by at least two methods. The resulting network is a synthesis of the altered mechanism: is smaller than the largest solutions (SigMod and dmGWAS), which makes it more manageable, and includes the majority of the strongly associated smaller solutions (SConES and heinz). The consensus subnetwork captures mechanisms and genes known to be related to cancer, recovering familial breast cancer genes as well as genome regions associated to cancer susceptibility. However, thanks to its small size and its network structure, it provides compelling hypotheses of non-canonical

mechanisms involved in carcinogenesis, like mitochondrial translation and chaperone activity.

In order to produce the consensus networks, we dealt with the different interfaces, preprocessing steps, and exhibited unexpected behaviors of the various methods. To facilitate that other authors apply them to new datasets and aggregate their solutions, we built six nextflow pipelines [44] with a consistent interface and, whenever possible, parallelized computation. They are available on GitHub: <https://github.com/hclimente/gwas-tools>. Importantly, those methods that had a permissive license were compiled into a Docker image for easier use, which is available on Docker Hub hclimente/gwas-tools.

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Supplementary materials

Table 1: Summary statistics on the results of SConES on the three SNP-SNP interaction networks. The first row within each block contains the summary statistics on the whole network.

| Network | SNPs | Edges | Subnetworks | $\overline{\text{betweenness}}$ | \hat{P}_{SNP} |
|-----------|--------|----------|-------------|---------------------------------|------------------------|
| GS | 197083 | 197060 | - | 2.03×10^7 | 0.49 |
| SConES GS | 1590 | 1585 | 5 | 2.52×10^7 | 0.023 |
| GM | 197083 | 6442446 | - | 3.99×10^6 | 0.49 |
| SConES GM | 1692 | 177611 | 5 | 4.40×10^6 | 0.055 |
| GI | 197083 | 28733720 | - | 1.46×10^6 | 0.49 |
| SConES GI | 408 | 539 | 5 | 9.33×10^6 | 0.076 |

Table 2: Summary statistics on the results of multiple network methods on the gene-gene interaction network. The first row contains the summary statistics on the whole network.

| Network | Genes | Edges | Mean betweenness | Median P_{gene} | Correlation _{consensus} |
|-----------|-------|-------|------------------|--------------------------|----------------------------------|
| SConES GS | 5 | 0 | 9805 | 2.7×10^{-5} | 0.19 |
| SConES GM | 28 | 2 | 4267 | 0.067 | 0.12 |

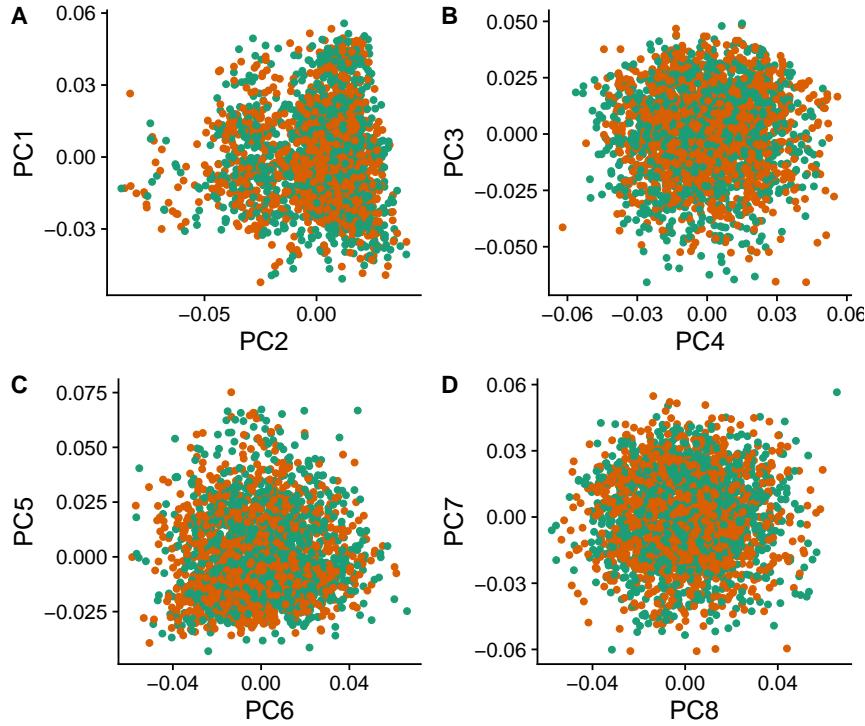


Figure 1: (A,B,C,D) Eight main principal components computed on the genotypes of GENESIS. Cases are colored in green, controls in orange.

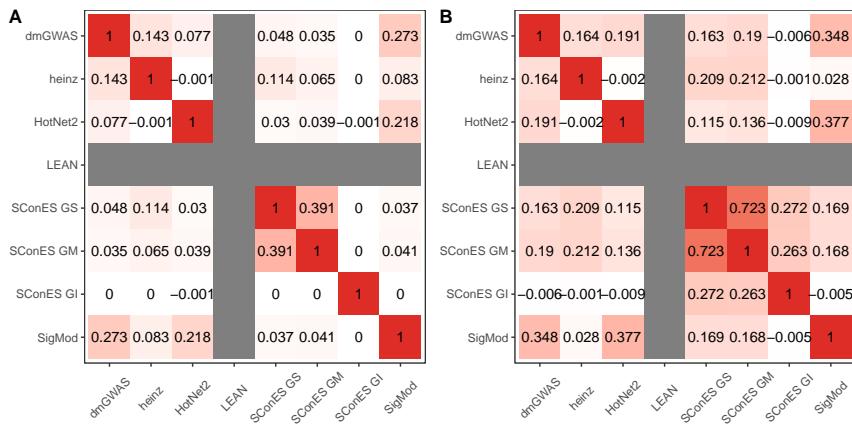


Figure 2: Pearson's correlation between the different solution gene subnetworks.

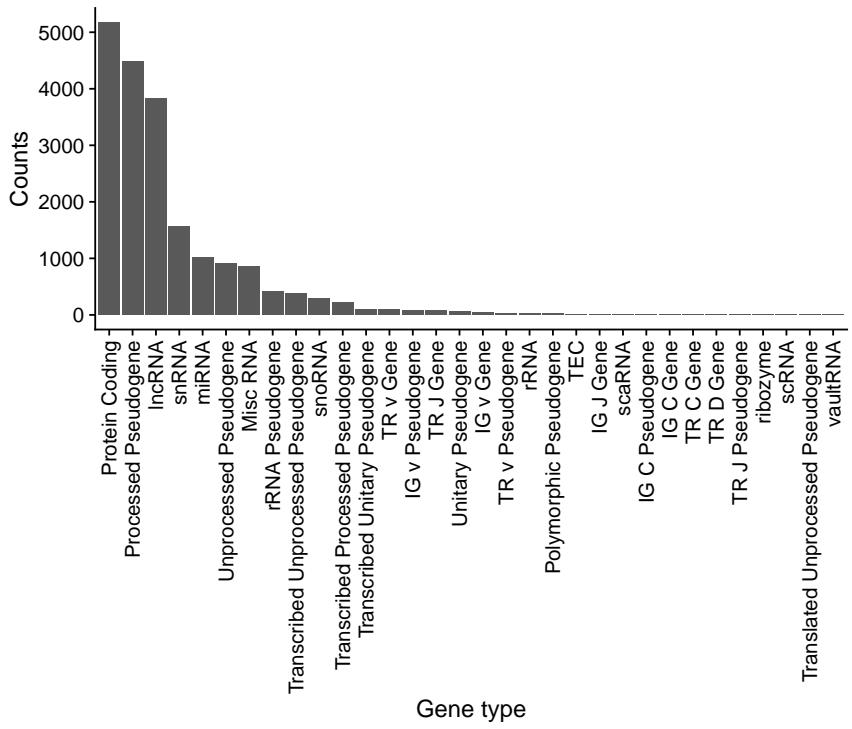


Figure 3: Biotypes of genes from the annotation that are not present in the HINT protein-protein interaction network.

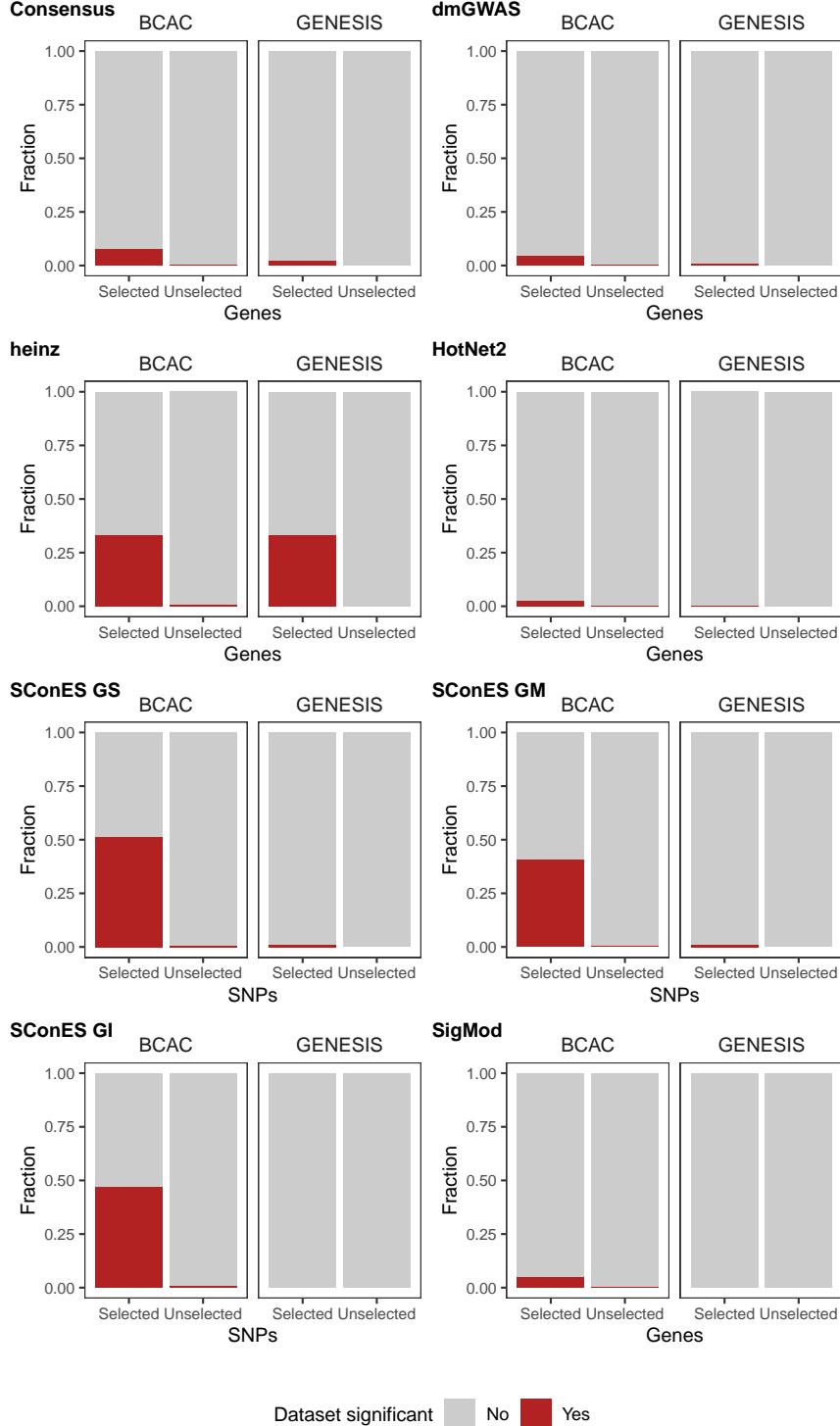


Figure 4: Bonferroni significance, in either the GENESIS or the BCAC datasets, of the genes (and SNPs in the case of SConES) detected by the network methods, and in the consensus subnetwork. LEAN was excluded, as it did not select any gene.

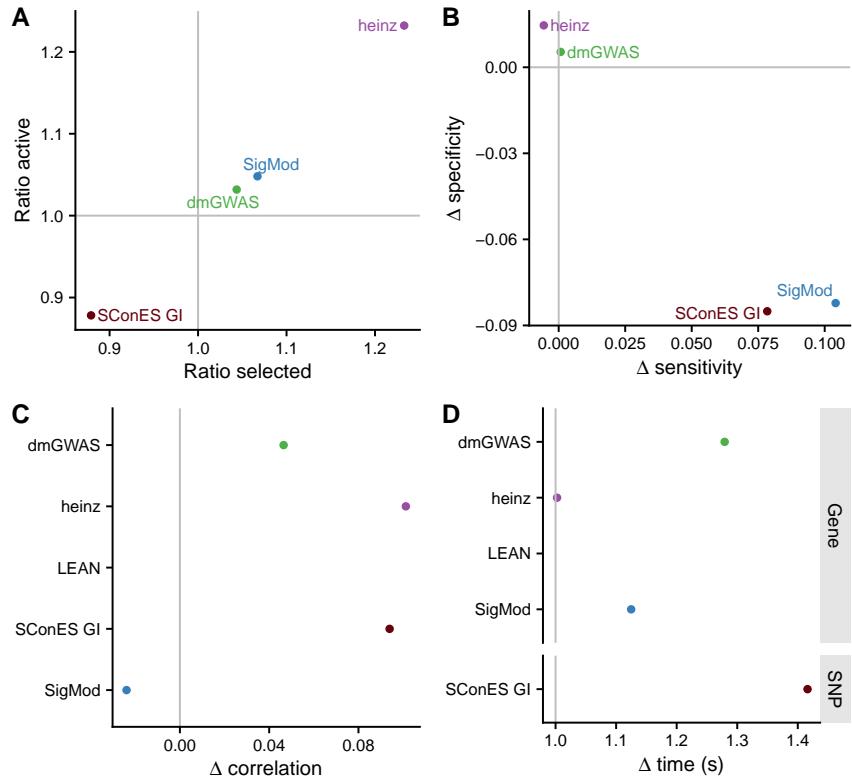


Figure 5: Comparison of benchmark on high-throughput interactions to benchmark on both high-throughput and literature curated interactions. Grey lines represent no change between the benchmarks (1 for ratios, 0 for differences). **(A)** Ratios of the selected features between both benchmarks and of the active set. **(B)** Shifts in sensitivity and specificity. **(C)** Shift in Pearson’s correlation between benchmarks. **(D)** Ratio between the runtimes of the benchmarks.

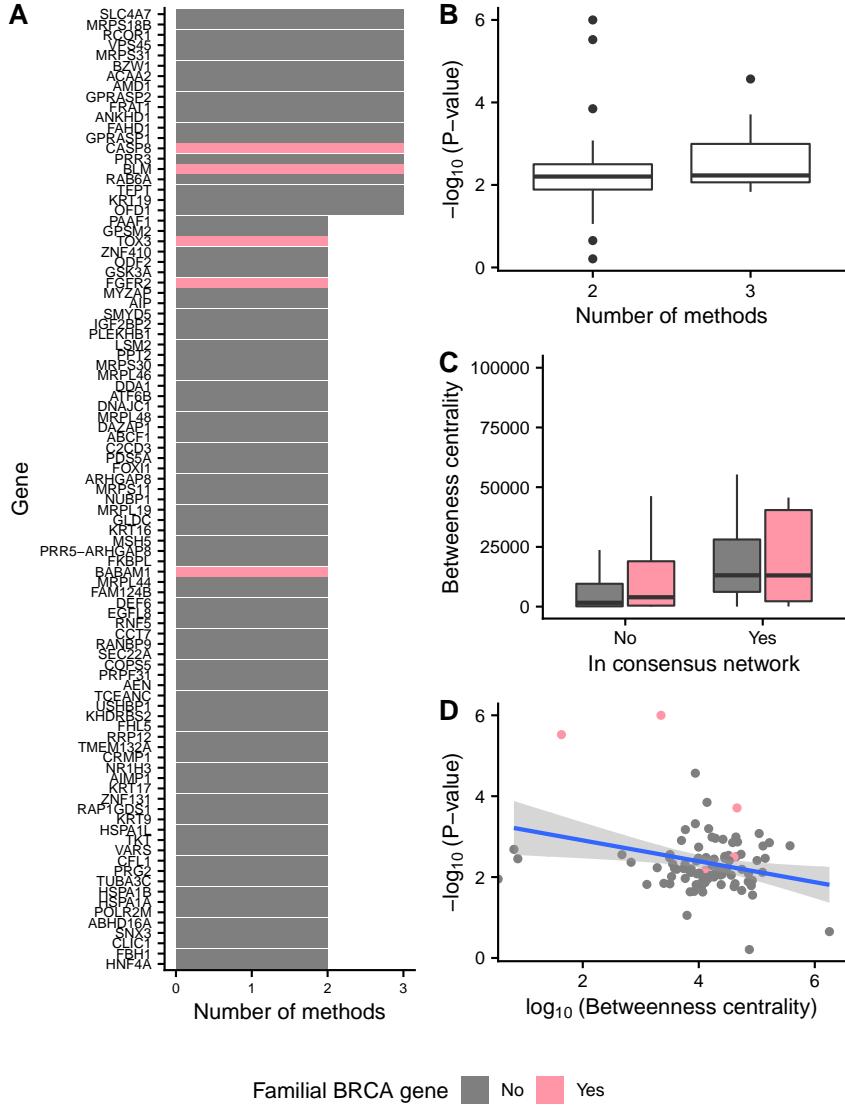


Figure 6: Genes on the consensus network. Familial breast cancer genes are colored in pink; the rest are colored in grey. **(A)** Number of methods selecting every gene in the subnetwork. **(B)** VEGAS P-values of association of the genes, with regards to the number of methods that selected them. **(C)** Comparison of betweenness centrality of the genes in the consensus network and the other genes in the PPIN and not in the consensus network. To improve visualization, we removed outliers. **(D)** Relationship between the \log_{10} of the betweenness centrality and the $-\log_{10}$ of the VEGAS P-value of the genes in the consensus network. The blue line represents a fitted generalized linear model.

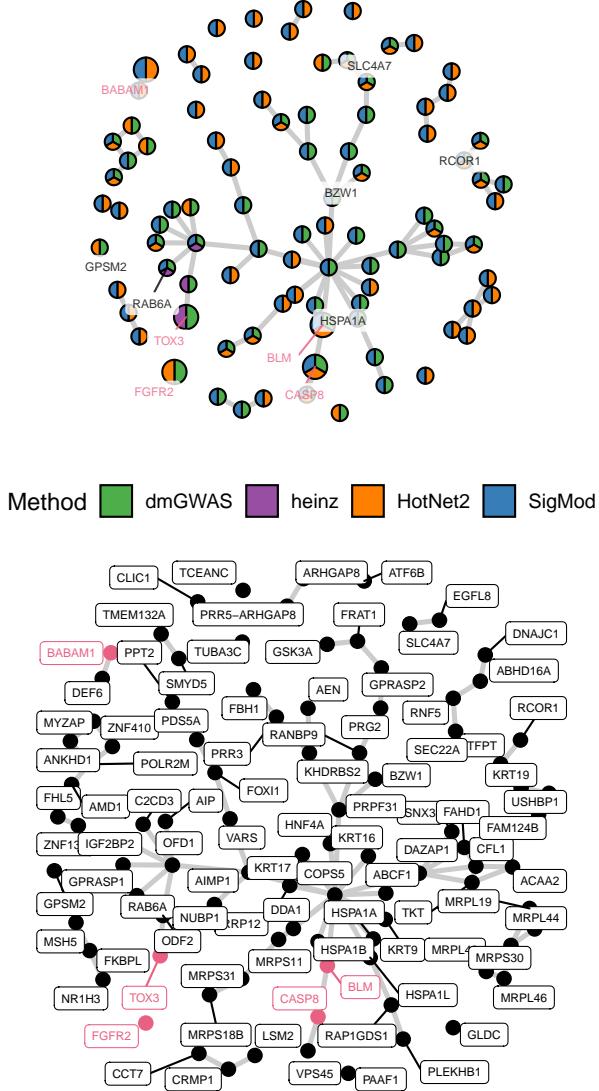


Figure 7: Consensus subnetwork on GENESIS (Section Consensus network). **(A)** Each node is represented by a pie chart, which accounts the methods that selected it. The labeled genes have a VEGAS2 P-value < 0.001 and/or are known familial breast cancer genes (colored in pink). This panel is equivalent to Figure 4. **(B)** The name of every gene is indicated.