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Supplementary Information Text and Table File 1 for: Network Analysis of the Genomic Basis of the Placebo Effect, Ms. # 93911-INS-RG-TR-2

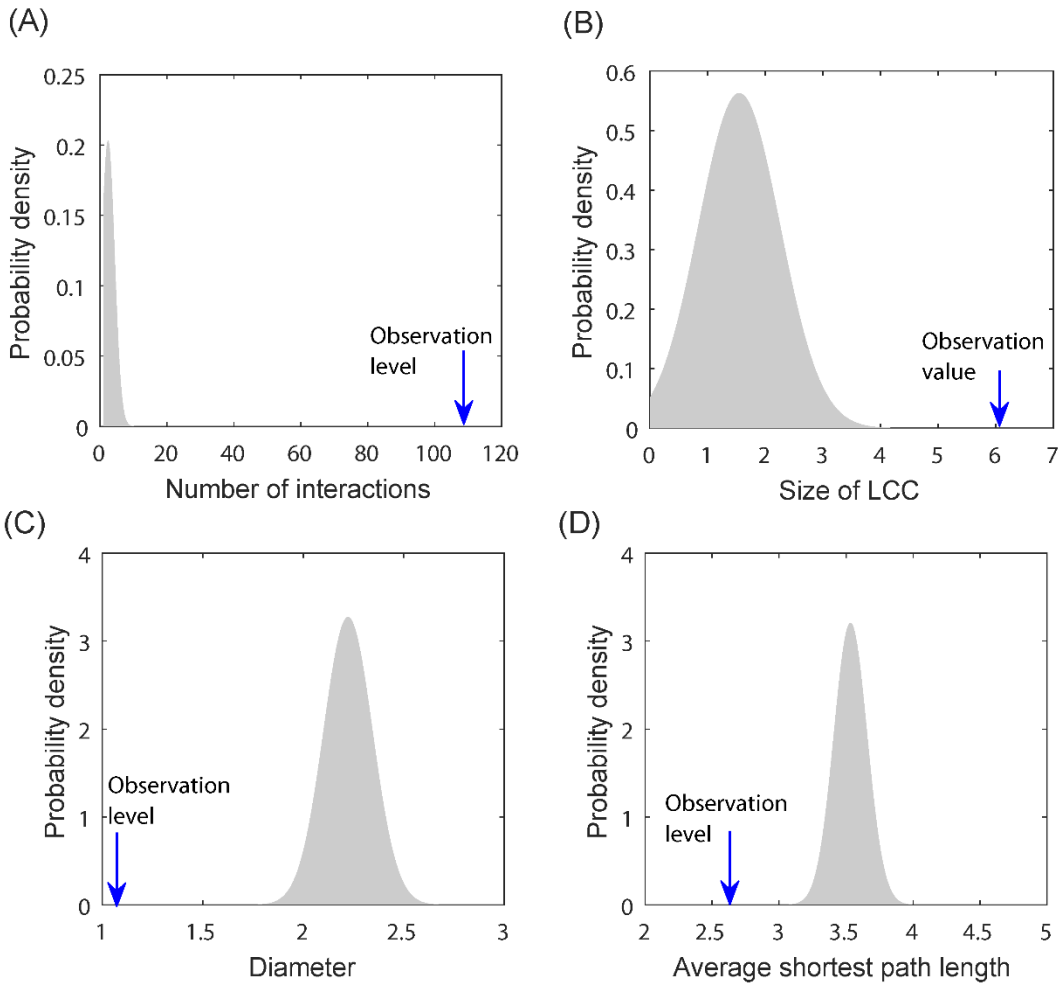

Figure S1. The topoligical properties of the placebome module. (A) The placebome module has significantly more interactions than random expectation ($p<1.0\times10^{-16}$). (B) The placebome module has a significantly larger LCC than random expectation ($p<1.0\times10^{-16}$). (C) The placebome module has a significantly smaller diameter than a random protein set ($p=4.5\times10^{-21}$). (D) The placebome module has a significantly smaller average shortest path length than a random protein set ($p=1.8\times10^{-13}$).

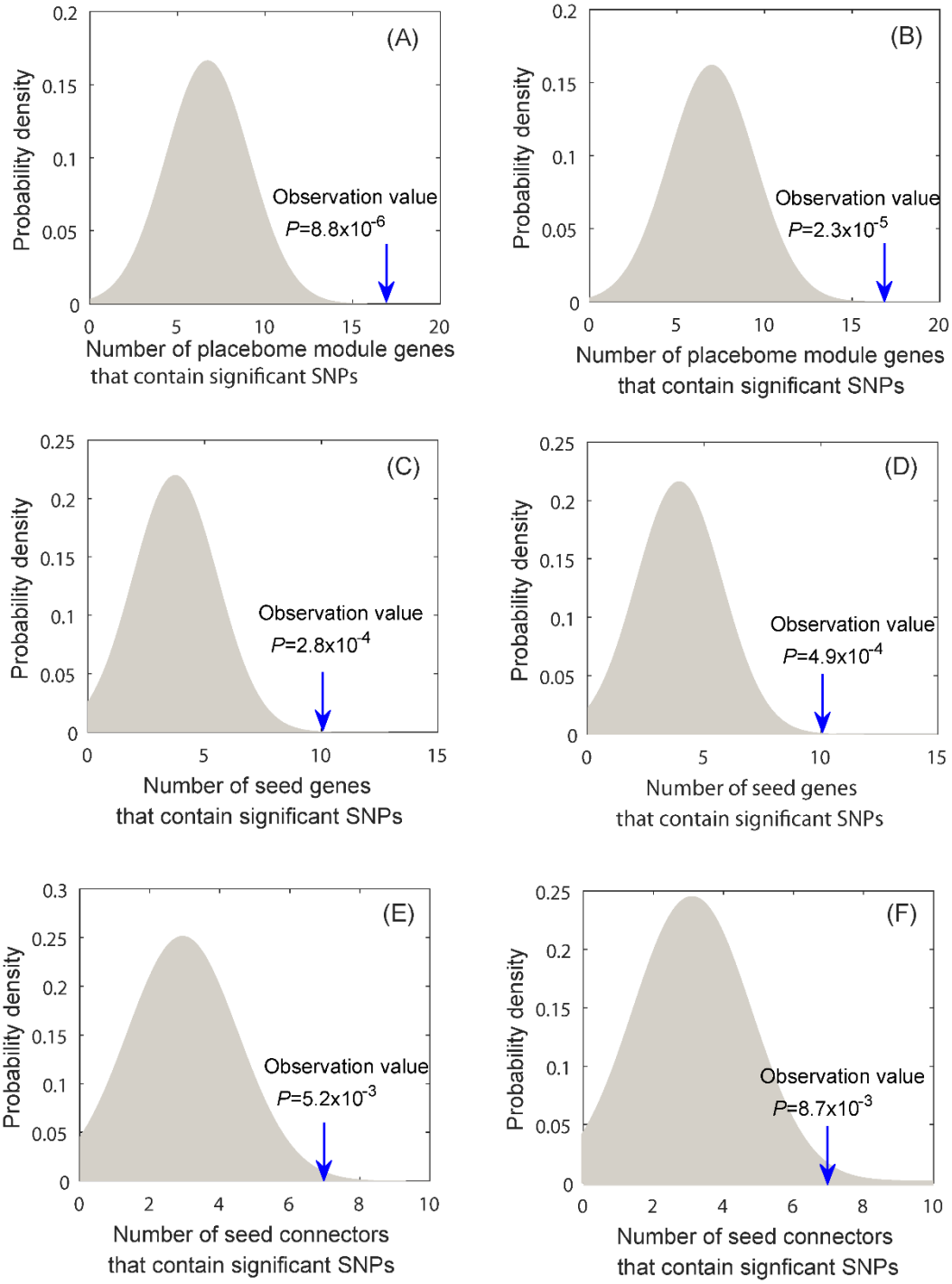
(A)
Probability density
Observation value
P=8.8x10-6
Number of placebome module genes
that contain significant SNPs
(B)
Probability density
Observation value
P=2.3x10-5
Number of placebome module genes
that contain significant SNPs
(C)
Probability density
Observation value
P=2.8x10-4
Number of seed genes
that contain significant SNPs
(D)
Probability density
Observation value
P=4.9x10-4
Number of seed genes
that contain significant SNPs
(E)
Probability density
Observation value
P=5.2x10-3
Number of seed connectors
that contain significant SNPs
(F)
Probability density
Observation value
P=8.7x10-3
Number of seed connectors
that contain signficant SNPs

Figure S2. Significant SNP enrichment of the placebome module. (A) Compared to a random gene set from the GWAS background, the placebome module is significantly enriched with SNPs that modify the outcome of the placebo arm. (B) Compared to a random gene set from the GWAS background mapped to the human interactome, the placebome module is significantly enriched with SNPs that modify the outcome of the placebo arm. (C) Compared to a random gene set from the GWAS background, the placebome seed genes are significantly enriched with SNPs that modify the outcome of the placebo arm. (D) Compared to a random gene set from the GWAS background mapped to the human interactome, the placebome seed genes are significantly enriched with SNPs that modify the outcome of the placebo arm. (E) Compared to a random gene set from the GWAS background, the placebome seed connectors are significantly enriched with SNPs that modify the outcome of the placebo arm. (D) Compared to a random gene set from the GWAS background mapped to the human interactome, the placebome seed connectors are significantly enriched with SNPs that modify the outcome of the placebo arm.

Compiling the comprehensive human interactome

The mechanisms underlying placebo responses may involve multiple types of molecular interactions. We, therefore, used a comprehensive human interactome, which combines physical macromolecular interactions from different sources, to ascertain the existence of a placebome module. The consolidated human interactome contains protein-protein interactions, protein complexes, protein-DNA interactions, kinase-substrate interactions, metabolic interactions, and signaling pathways. The protein-protein interactions are derived from several high-throughput yeast-two-hybrid studies (*1-4*) and have also been combined with binary interactions from IntAct and MINT databases (*5, 6*), as well as literature-curated interactions obtained from low throughput experiments reported in the IntAct, MINT, HPRD, and BioGRID databases (*5-8*). The manually curated dataset of mammalian protein complexes (CORUM) and experimentally determined human protein complexes are also included in the comprehensive set of protein-protein interactions (*9, 10*). Protein-DNA regulatory interactions are taken from the TRANSFAC database (*11*), and kinase-substrate interactions are obtained from the PhosphositePlus database (*12*). Metabolic enzyme-coupled interactions (two enzymes that share adjacent reactions) are derived from the KEGG and BiGG databases as compiled previously (*13*). In addition, protein interactions from 3D structural prediction and signaling interactions are also included in the construction of the interactome (*14, 15*). This consolidated human interactome has 14,174 proteins (nodes) with 170,303 interactions (edges), after removing duplicate interactions and self-loops. All of the placebome seed gene products in Table 1 can be found in this human interactome.

Connecting the placebome seed proteins

Owing to the incompleteness of placebome seed genes and of the human interactome itself, these seed gene products may not be densely connected to each other to form a network module. We, therefore, developed an algorithm in which we attempt to connect the placebome seed proteins by using as few extra nodes as possible. The principle underlying this algorithm is that seed genes should not be very far from each other and, thus, should reach each other through very short paths. The algorithm, called the Seed-Connector algorithm, is iterative as follows:

Step 1. Assume that the seed genes induce a subnetwork. Calculate the size of the largest connected component (LCC) of the subnetwork.

Step 2. Consider all the interactors of the seed genes as identified from the human interactome. Add each interactor temporarily to the seed gene list one-by-one. Obtain the subnetwork induced by this temporary seed gene list and determine the size of its LCC.

Step 3. Select those interactors that can increase the coverage of seeds in the LCC of the subnetwork maximally, and add them to the seed gene list.

Repeat Step 1, Step 2, and Step 3 until none of the interactors can increase the coverage of seed genes in the LCC of the induced subnetwork. The final subnetwork is the predicted placebome module, which is obtained by including as few additional nodes as possible. The placebome module obtained by this algorithm has a very high ratio of seed genes (gene products) to connector genes (gene products).

Disease modules and drug categories

To gain insights into placebo responses in different diseases, we collected a list of 20 'benchmark' diseases (18 known to have moderate or high placebo responses and 2 known to have little or no placebo response) and 5 symptom phenotypes on which we have prior knowledge about the placebo response based on the literature, and obtained their associated genes from Phenopedia in the HuGE navigator (*16*). The benchmark diseases with high responses are alcoholism (*17*), anxiety (*18*), asthma (*19*), Crohn disease (*20*), depression (*21*), diabetic neuropathies (*22*), duodenal ulcer (*23*), epilepsy (*24*), eating disorders (*25*), fibromyalgia (*22*), irritable bowel syndrome (*26*), Parkinson disease (*27*), migraine disorders (*28*), osteoarthritis (*29*), chronic pancreatitis (*30*), restless leg syndrome (*31*), schizophrenia (*32*), and ulcerative colitis (*33*). The two with little or no responses are hepatocellular carcinoma and renal cell carcinoma (*34*). The five symptoms we considered are pain (*35*), headache (*36*), nausea (*37*), fatigue (*38*), and hot flashes (*39*). To obtain a reliable list of associated disease genes, we only considered those with at least two publications that support the association if the number of associated genes is over 100.

For prediction purposes, we obtained a comprehensive list of diseases from Phenopedia in the HuGE navigator (*16*) and assessed the relationships between the placebome module and these diseases at the systems level. There are 2909 Medical Subject Headings (MeSH) disease terms (downloaded in May, 2016), among which we only consider the terms with more than 50 associated genes. We also removed some terms that are not typical diseases or symptoms. A final list of 859 diseases (and symptoms) were considered in our analysis. Again, we only considered those associated genes with at least two publications that support the association if the number of associated genes is over 100. We mapped the disease- or symptom-associated gene products to the human interactome and derived disease modules or symptom modules.

We collected drug categories from DrugBank (*40*) in which drugs are categorized into different groups based on their therapeutic indications. The targets of these drugs include therapeutic drug targets, drug carriers, drug enzymes, and drug transporters. We collectively refer to this compilation as drug targets. Drug targets are classified based on their drug categories and mapped to the human interactome. To increase the power of prediction, we only considered those drug categories with at least 20 drug targets in the human interactome. A total of 193 drug categories satisfy this criterion. We then assessed the relationships between the placebome module and drug target module from each category at the network level.

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