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Abstract

Statistical association studies have contributed significantly in the detection of novel genetic factors associated with complex diseases. However, there are various challenges in solving the missing heritability issue solely depending on statistical evidence of the association between genotype and phenotype, especially for sequencing data. Incorporation of biological information that reflects the complex mechanism of disease development is likely to increase the power of association studies for detecting novel disease genes. In this study, we develop a statistical framework for association studies that integrates the information of the functional effect of SNPs to the disease related protein-protein interactions. The method is applied to GAW19 exome sequencing data of uncorrelated individuals for detecting novel genes associated to hypotension. Based on both the real and simulated phenotypes of hypertension, the method is compared with multiple well-known association tests for sequencing data.

Acknowledgement

Without the help from Dr. Zheyang Wu, Dr. Dmtry Korkin, and Hongzhu Cui, the completion of this project would not have been possible. Thanks to their help of providing guidelines, sharing techniques and contributing to the results of this project. I would like to thank the Genetic Analysis Workshop for providing the Sequence, Blood Pressure and Expression Data.

# Chapter 1: Introduction

Statistical association studies serve an important role in finding putative disease genes for further biological validation and reasoning. There are many association methods, but the common essential idea is to test the strength of statistical evidence for abnormal / non-random mutation distribution in cases and in controls. Such statistical evidence won’t be strong or reliable if data sample size is relatively small and/or SNP mutations are rare, often the challenges imposed in current sequencing studies. Furthermore, the mechanism of genetic effect is complex, not a straight line from DNA to disease. A gene may be critical to a disease pathway, but the final disease status are affected by many other factors so that the association evidence measured strictly by genotype and phenotype data could also be weak. This is the challenge for finding more subtle disease genes to explain the missing heritability, especially after the low-hanging fruits have been picked. In order to address these challenges and increase the statistical power of association studies, it is promising to properly integrate biological prior information of the variants that reflect the middle steps of the genetic mechanism to disease development process.

In this study, we develop a statistical framework that allows incorporating prior information of SNPs into association tests. The basic idea is to prioritize SNPs that show prior importance to disease development. This is realized by relatively weighting the genotype of SNPs according to their prior information in testing a SNP set, which is treated as the functional unit of association, e.g., a gene. This framework has two major components. First, SNP weights are properly generated based on their functional annotation. Second, a likelihood ratio test (LRT) is constructed to incorporate these weights. To detect the presence of disease SNPs in a gene, LRT statistic is the ratio of the likelihoods between the null and the alternative regarding to whether the distribution in cases differ that in controls. Thus LRT is flexible to construct according to the meanings of the null of no association and the alternative while incorporating informative weights. Furthermore, LRT is optimal for detecting weak and sparse signals {Ingster, 1997 #2436;Yang, 2014 #2444}. There are different versions of LRT, but we adapt a formulation by Chen et al. {Chen, 2013 #2486} as the prototype statistic for sequencing data.

Protein-protein interactions (PPI) are one important component related to disease development. Disease gene may function through influencing PPIs. Several recent genome-wide association studies (GWAS) have reported the value of incorporating PPI information into the pipeline of identifying novel genes of Type 1 diabetes and kidney dysfunction {Bergholdt, 2012 #2484;Chasman, 2012 #2487}. However, their methodology mainly uses generic functional information, *e.g.*, GO terms, to filter candidate genes and SNPs for test, but the association test itself is traditional without incorporating such information {Consortium, 2007 #2909}. Filtering will loosen the strict genome-wide significance level in favour of relatively weak association signals of true functional SNPs and genes, but it would be nicer to drop as few genes as possible, and combine the prior biological information with association test process in a quantitative fashion. In this study, we predict and annotate the effect of SNPs to disease related PPIs. Then we convert the annotation into a component of SNP weighting scheme for incorporating into the LRT test. The purpose is not to restrict the gene candidates but to use PPI information to improve the ranking profile of all genes.

# Chapter 2: Methodology

## Research design

To implicitly incorporate the PPI information for aiding gene hunting, we estimate and employ the weighting scheme of variants according to their effects on PPIs and study their group association by using a flexible LRT testing procedure. Figure 1 shows the workflow of four steps to achieve the goal. First, we determine the seed genes associated with Hypertension based on literature, data evidence and other related sources ([Welter, D. et al, 2014](#Welter); [Online Mendelian Inheritance in Man, OMIM](#Online)). Second, we analyse PPI network (physical interactions only) centering on these seed genes to find out candidate genes associated with Hypertension. The candidate genes will be utilized to study the statistical association with Hypertension ([Szklarczyk, Damian, et al, 2011](#Szklarczyk)). Third, we create annotations for SNPs regarding their impact on the several aspects of PPI networks. Fourth, we incorporate the SNP annotation information (weighting score) into the statistical association study by the LRT test. In the following, we give details of each step.

## Seed gene detection

The “seed genes” are obtained from OMIM (Online Mendelian Inheritance in Man) by 1) Searching the key word: hypertension OR (("high blood pressure")), and 2) Extracting only genes with direct “phenotype- gene relationships”. The original gene list from step (1) yielded 290 genes. Step (2) is performed by manually justifying each gene and taking only the entries with “phenotype-gene relationship” recorded on OMIM. See result section for a list of 21 genes that was obtained as the final seed genes.

## Candidate gene detection

The “candidate genes” are obtained from multiple sources. First, HINT (High-quality INTeractomes) from Yulab, is a database containing high-quality PPIs, which are complied from various sources. It takes gene(s) as input and output an interactive image of its PPI network. We input seed genes into HINT, select interaction type “Binary”/ “co-complex” respectively, and enable the high-quality filter to obtain only high-quality interactomes. The genes in the PPI network are considered as candidate genes. [See the figure for seed genes and their neighbourhood genes.]

**Second, Human Interactome Database (**[**http://interactome.dfci.harvard.edu/index.php?page=home**](http://interactome.dfci.harvard.edu/index.php?page=home)**)** is part of the CCSB project, which takes efforts to map the human binary interactome. Their long-term goal is to generate and analyze high-quality yeast two-hybrid (Y2H) interactions at high-throughput for all pairwise combinations of predicted gene products for which there is at least one Gateway-cloned ORF available (Human ORFeome website). All individual datasets, including our most recent unpublished data, are described below with hyperlinks to the prepublication data or the relevant publications.

Third, t**he association Test C(α)** enables the test for rare variants disease association, under the assumption that the rare variants in cases and controls are a mix of deleterious, protective and neutral variants. The C(α) statistic is computed as follows:

The C(α) test would yield a list of variants with considerably low p-values, which could be considered as candidate genes. The cut-off of p-value for C (α) test is set to 0.001.

**Fourth, t**he association test CMC (Combined and Multivariate Collapsing method for rare variants) tests for rare variants disease association as well. CMC method groups variants by gene. The CMC method uses Fisher’s test statistic to avoid the computationally intensive permutation procedure. The input would be our raw data. Output variants with low p-values would be considered as candidate genes. The cut-off of p-value for CMC test is set to 0.002.

## Annotate nsSNVs according to protein binding sites

We annotated non-synonymous SNVs by sequence-based prediction on whether they locate in protein binding sites. In particular, based on the variant information provided in the VCF files of the odd numbers of chromosomes for uncorrelated individuals, applying ANNOVAR [citation], we annotated 4,457 nsSNVs on 2,711 genes regarding to whether they are located on protein binding sites or not.

## Likelihood ratio tests (LRT)

For a gene (or a functional group of SNP set), the generic LRT formula is

(1)

where , , and are the likelihoods for the distributions of an appropriate disease-association measure in cases, in controls, and in both groups. The numerator of LRT separates the likelihoods in cases and in controls to model the alternative hypothesis that there exists an association in terms of the differentiation between the two groups; the denominator pools the data of cases and controls together for the likelihood of the null hypothesis of no association. Here we adapt an LRT based on Bernoulli likelihoods {Chen, 2013 #2486}:

, (2)

where and are the number of cases and controls, , and (, and ) are the total numbers (and the corresponding estimated proportions) of the burden scores that exceed a threshold in cases, controls and both groups, respectively.

The burden scores are the collapsed genotypes over SNVs on a gene, which measure the overall mutation distributions {Morgenthaler, 2007 #2847}. Specifically, for the th gene of the individual , the burden score is

(3)

where is the genotype of SNP of individual . We search a sequence of threshold and choose the value that maximizes the test statistic. When ≤ , the test statistic is adjusted to be

. (4)

We apply permutation-based test to calculate p-values, which accommodates the departure of assumptions not necessarily satisfied in real data to control the type I error rate. We implemented the LRT and permutation test by R functions, and applied Variant Tools {San Lucas, 2012 #2441;Wang, 2014 #2440} to manipulate data and to call these R functions.

## LRT for burden score weighted by binding site information (LRT-BS)

This modified LRT method similar as above but with a weighted burden score

(5)

where is the weight to indicate the importance of variants according to whether they are located on protein binding sites.

# Chapter 3: Results

## Simulation data analysis

GAW19 exome sequencing variants on odd numbers of chromosomes for uncorrelated individuals are used as the genotype data. Phenotypes are the hypertension status (about 336 cases and 1,607controls) of the 200 simulations. Using the simulated data, we can study the statistical power and type I error rate control of the association tests.

First, we study the strategy to weight the functional SNVs. Figure 1 shows the power curves of three genes containing simulated functional SNVs: SPTBN4 (functional SNVs have relatively strong beneficial effects), TPR (functional SNVs have relatively weak effects), and TCIRG1 (functional SNVs have relatively strong deleterious effects). We arbitrarily select 10 functional SNVs and assign weight (2, 5 or 10), the rest functional SNVs are pretended unknown, and they and the rest irrelevant SNVs are assigned weight (1 or -1). Figure 1 shows that different weighting schemes perform differently, but in general the combination 1-10 is relatively good and robust. Thus, we assign potentially functional SNVs and the rest weights that have the same sign but with relatively big difference. For the nsSNVs in binding site, we assign weight 10; for nsSNVs on in binding site, we assign weight 5; and for the synonymous SNVs, we assign weight 1.

Second, we compare LRT and the LRT-BS regarding to their statistical power and size.

## Real data analysis

The phenotype data is based on the real DBP and SBP, for which hypertension cases are defined as individuals with SBP>=140 or DBP>= 90 according to the American Heart Association. Thus for the real data we have 394 cases and 1,457 controls.

Four methods are applied to the association tests for genes (RefGene): C-α test {Neale, 2011 #2850}, CMC test {Li, 2008 #2822}, LRT, and LRT-BS. Table 1 shows the top genes (with p-values < 2.0E-4) yielded by C-α test (4 genes), CMC (3), LRT (5), LRT-BS (8). At the same level of the genomic inflation factor λ, LRT-BS has three more genes discovered than LRT, which include gene MSL2, a gene also discovered by CMC with a good inflation level of λ=1.03. Thus LRT-BS is more powerful than LRT.

# Chapter 4: Discussions

One of the limits of current method is that genomic inflation factor is relatively big, which needs further research and modification.

The annotations for whether a SNV is on the binding site has limit in specifying the influence of the SNVs to the protein-protein interactions. Further annotations on the SNV effect (beneficial, deleterious, or neural) to individual PPIs and the PPI network as a whole will provide more information. LRT has the potential to incorporate different levels of the information by constructing multiple components of the test statistics. We will test on this idea later as long as we get the improved estimation of the SNV effects.

# Chapter 5: Conclusions and Justification

Biological information on SNVs can be used to improve the association tests. We incorporate the information on whether nsSNVs are located in protein binding site into a LRT test, which has the similar genomic inflation factor as the original LRT test but provide more power to detect more significant genes. Among the putative genes discovered by mutlple methods, MSL2 are discovered by both CMC and LRT-BS, and is worth further validation study.

# References

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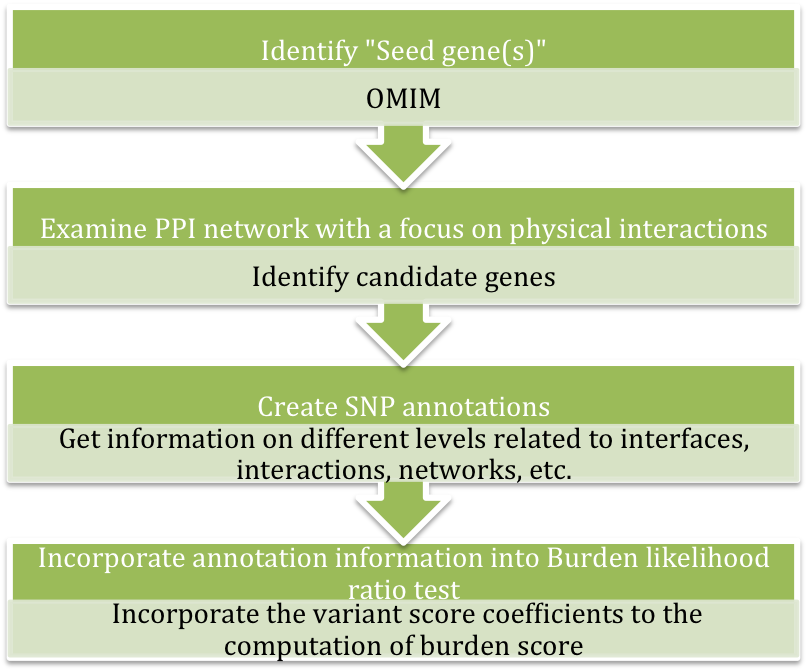
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Welter, D., MacArthur, J., Morales, J., Burdett, T., Hall, P., Junkins, H., ... & Parkinson, H. (2014). The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic acids research*, *42*(D1), D1001-D1006.

# Figures

## Figure 1 - Gene detection working flow.

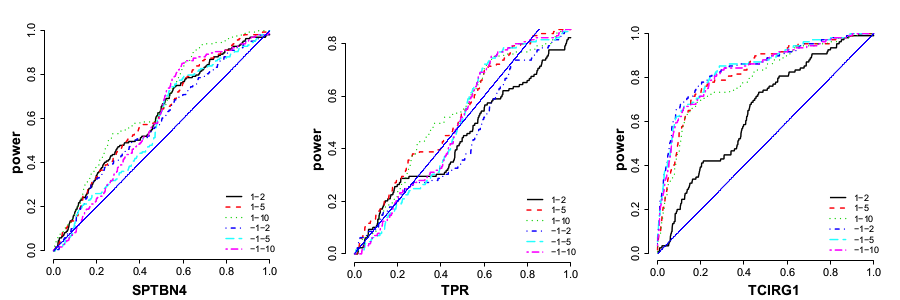
Four steps are involved in gene detection. Two main components: Estimation of SNP annotations on their effects to PPI; Association test by LRT.



## Figure 2 - QQ-plot for CMC, C-alpha, LRT methods



## Figure 3 - Systematic weighting experiment for simulated gene: SPTBN4, TPR and TCIRG1.



# Tables

## Table 1 - Seed gene list

Top genes and their p-values by four association tests (λ is the genomic inflation factor).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Genes | Chr | C-α  (λ=1.11) | CMC (λ=1.03) | LRT (λ=1.27) | LRT-BS (λ=1.28) |
| C7orf55-LUC7L2 | 7 | 1.99E-04 | - | - | - |
| LUC7L2 | 7 | 1.99E-04 | - | - | - |
| DNAH9 | 17 | 1.99E-04 | - | - | - |
| AKAP8 | 19 | 1.99E-04 | - | - | - |
| MSL2 | 3 | - | 4.39E-05 | - | < 1.00E-4 |
| ZBTB4 | 17 | - | 9.21E-05 | - | - |
| BCHE | 3 | - | 1.55E-04 | - | - |
| LZIC | 1 | - | - | < 1.00E-4 | < 1.00E-4 |
| ABO | 9 | - | - | - | - |
| COL15A1 | 9 | - | - | < 1.00E-4 | < 1.00E-4 |
| PSPC1 | 13 | - | - | - | < 1.00E-4 |
| TLN2 | 15 | - | - | - | < 1.00E-4 |
| ZNF557 | 19 | - | - | - | < 1.00E-4 |
| ITGA2 | 5 | - | - | < 1.00E-4 | - |
| ZMYM5 | 13 | - | - | - | < 1.00E-4 |
| YIPF2 | 19 | - | - | < 1.00E-4 | < 1.00E-4 |
| PAEP | 9 | - | - | < 1.00E-4 | - |

# Appendix

## weightBurdenTest\_invDir.R

# BEGINCONF

# [geneSize]

# [permuN]

# [pvalue]

# ENDCONF

#Note this function give the inversed direction weight, contrasting to weightBurdenTest.R

#######################################################

#Description: Interface R function to be called by Variant Associate Tools. It has the choice to calculate an association test statistic based on direction weighted burden score. That is, if MAF in cases < MAF in controls, direction weight = 1, o.w., =-1. Further explicit weights can be multiplied by these direction weights to get the final weights. Permutation test is used to get p-values. Direction weights are re-calculated after each permutation.

#Depends on:

#Arguments: dat: data from Variant Tools. Require: (a) For phneotype, 1s indicate cases; 0s indicate controls. (b) genotype scores are counts of the minor alleles (mutations).

# Yphenotype.name: Name of the phenotype

# testFunc: The name of the association test statistic calculating function

# weightScheme: "noWei": no weighting; "expWei": explicit weight only; "dirWei": direction weight only; "expDirWei": explicit weights are multiplied b direction weights to get the final weights. Default is no weighting. Variant Tools command must specify the expliciate weight for "expWei" and "expDirWei";

# permuN: Number of the permutations

#Details:

#Value: Size of snp group; # of permutations; pvalue

#Note:

#Author(s): ZWu, DZhang

#References:

#See Also:

#Example:

#######################################################

weightBurdenTest\_invDir = function (dat, phenotype.name = "HTN", testFunc="LRTStat", weightScheme="noWei", permuN = 5000) {

phenotypes = dat@Y[,phenotype.name]; #phenotype

caseN = sum(phenotypes==1);

controlN = sum(phenotypes==0);

genotypes = dat@X; #Genotype

genotypes[is.na(genotypes)] = 0; #Missing genotype are treated as the major allele.

#genotypes= round(genotypes);

#calculate direction weights based on genotypes and phenotypes

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes))); #no weighting

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V; #explicit weights

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[phenotypes==1])/(2\*caseN);

maf.contr = sum(g[phenotypes==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

#For real data

stat = do.call(testFunc, list(phenotypes, genotypes, weights, caseN, controlN));

#For permutation test

stat.perms = 0;

for (i in 1:(permuN/10)) {

pheno.perm = sample(phenotypes); #Permutation stage 1

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes)));

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V;

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[pheno.perm==1])/(2\*caseN);

maf.contr = sum(g[pheno.perm==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

stat.perms[i] = do.call(testFunc, list(pheno.perm, genotypes, weights, caseN, controlN));

}

pValue = (sum(stat.perms >= stat)+0.5)/(length(stat.perms)+1);

if (pValue > 0.1) {

return (list(geneSize=ncol(genotypes), permuN=i, pvalue=pValue));

}else {

for (i in (permuN/10+1):permuN) { #Permutation stage 2

pheno.perm = sample(phenotypes);

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes)));

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V;

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[pheno.perm==1])/(2\*caseN);

maf.contr = sum(g[pheno.perm==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

stat.perms[i] = do.call(testFunc, list(pheno.perm, genotypes, weights, caseN, controlN));

}

pValue = (sum(stat.perms >= stat)+0.5)/(length(stat.perms)+1);

return (list(geneSize=ncol(genotypes), permuN=i, pvalue=pValue));

}

}

#######################################################

#Description: Calculate the LRT statistic according to Bernoulli distribution. The burden score can be weighted. The LRT statistic is gotten at the best burden score threshold that maximizes the statistic.

#Depends on:

#Arguments: phenotypes: A vector of binary phenotype values. Require: 1s indicate cases; 0s indicate controls.

# genotypes: Genotype data frame (cols: variants; rows: sample subjects)

# weights: A one-col data frame vector of variant weights wrt to genotypes. Default: all 1s (no weighting).

# caseN: Number of cases;

# controlN: Number of controls.

#Details:

#Value: LRT statistic

#Note:

#Author(s): ZWu, DZhang

#References: Chen, ..., Karchin 2013 PloS Paper

#See Also:

#Example:

#######################################################

LRTStat <- function(phenotypes, genotypes, weights=data.frame(rep(1, ncol(genotypes))), caseN, controlN)

{

burdenScores = data.matrix(genotypes) %\*% data.matrix(weights);

thresholds = c(unique(burdenScores), max(burdenScores)+1);

stats = array(0, dim=c(length(thresholds )));

for (i in 1:length(thresholds ))

{

Ys = (burdenScores >= thresholds[i]);

Ycase = sum(Ys[phenotypes==1]);

Ycontrol = sum(Ys[phenotypes==0]);

stats[i] = LRTStat.condi(Ycase, Ycontrol, caseN, controlN);

}

finalStat = max(stats[!is.na(stats)]);

return(finalStat);

}

#######################################################

#Description: Calculate the LRT statistic, conditional on given numbers of 1's in cases and controls

#Depends on:

#Arguments: Ycase: number of 1's in cases

# Ycontrol: number of 1's in controls.

# caseN: Number of cases;

# controlN: Number of controls.

#Details:

#Value: LRT statistic

#Note:

#Author(s): ZWu, DZhang

#References: Chen, ..., Karchin 2013 PloS Paper

#See Also:

#Example:

#######################################################

LRTStat.condi <- function(Ycase, Ycontrol, caseN, controlN)

{

pA = (Ycase + 1)/(caseN + 2);

pU = (Ycontrol + 1)/(controlN + 2);

p = (Ycase+ Ycontrol + 2)/(caseN+ controlN+ 4);

if (pA >= pU)

{

statistic = Ycase\*log10(pA)+(caseN-Ycase)\*log10(1-pA)+Ycontrol\*log10(pU)+(controlN-Ycontrol)\*log10(1-pU)-(Ycase+Ycontrol)\*log10(p)-(caseN+controlN-Ycase-Ycontrol)\*log10(1-p);

}

else

{

statistic = Ycase\*log10(pU)+(caseN-Ycase)\*log10(1-pU)+Ycontrol\*log10(pA)+(controlN-Ycontrol)\*log10(1-pA)-(Ycase+Ycontrol)\*log10(p)-(caseN+controlN-Ycase-Ycontrol)\*log10(1-p);

}

return(statistic);

}

## weightBurdenTest\_invDir\_ToGetTypeIErrorCurve.R

# BEGINCONF

# [geneSize]

# [permuN]

# [pvalue]

# ENDCONF

#Note this function give the inversed direction weight, contrasting to weightBurdenTest.R

#######################################################

#Description: Interface R function to be called by Variant Associate Tools. It has the choice to calculate an association test statistic based on direction weighted burden score. That is, if MAF in cases < MAF in controls, direction weight = 1, o.w., =-1. Further explicit weights can be multiplied by these direction weights to get the final weights. Permutation test is used to get p-values. Direction weights are re-calculated after each permutation.

#Depends on:

#Arguments: dat: data from Variant Tools. Require: (a) For phneotype, 1s indicate cases; 0s indicate controls. (b) genotype scores are counts of the minor alleles (mutations).

# Yphenotype.name: Name of the phenotype

# testFunc: The name of the association test statistic calculating function

# weightScheme: "noWei": no weighting; "expWei": explicit weight only; "dirWei": direction weight only; "expDirWei": explicit weights are multiplied b direction weights to get the final weights. Default is no weighting. Variant Tools command must specify the expliciate weight for "expWei" and "expDirWei";

# permuN: Number of the permutations

#Details:

#Value: Size of snp group; # of permutations; pvalue

#Note:

#Author(s): ZWu, DZhang

#References:

#See Also:

#Example:

#######################################################

weightBurdenTest\_invDir\_ToGetTypeIErrorCurve = function (dat, phenotype.name = "HTN", testFunc="LRTStat", weightScheme="noWei", permuN = 5000) {

phenotypes = dat@Y[,phenotype.name]; #phenotype

pehnotypes = sample(phenotypes); ##To get type I error even for functional genes

caseN = sum(phenotypes==1);

controlN = sum(phenotypes==0);

genotypes = dat@X; #Genotype

genotypes[is.na(genotypes)] = 0; #Missing genotype are treated as the major allele.

#genotypes= round(genotypes);

#calculate direction weights based on genotypes and phenotypes

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes))); #no weighting

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V; #explicit weights

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[phenotypes==1])/(2\*caseN);

maf.contr = sum(g[phenotypes==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

#For real data

stat = do.call(testFunc, list(phenotypes, genotypes, weights, caseN, controlN));

#For permutation test

stat.perms = 0;

for (i in 1:(permuN/10)) {

pheno.perm = sample(phenotypes); #Permutation stage 1

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes)));

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V;

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[pheno.perm==1])/(2\*caseN);

maf.contr = sum(g[pheno.perm==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

stat.perms[i] = do.call(testFunc, list(pheno.perm, genotypes, weights, caseN, controlN));

}

pValue = (sum(stat.perms >= stat)+0.5)/(length(stat.perms)+1);

if (pValue > 0.1) {

return (list(geneSize=ncol(genotypes), permuN=i, pvalue=pValue));

}else {

for (i in (permuN/10+1):permuN) { #Permutation stage 2

pheno.perm = sample(phenotypes);

if (weightScheme %in% c("noWei", "dirWei")) weights = data.frame(rep(1, ncol(genotypes)));

if (weightScheme %in% c("expWei", "expDirWei")) weights = dat@V;

if (weightScheme %in% c("dirWei", "expDirWei")) {

DirectionWeight = unlist(apply(genotypes, 2, function(g) {

maf.case = sum(g[pheno.perm==1])/(2\*caseN);

maf.contr = sum(g[pheno.perm==0])/(2\*controlN);

#ifelse(maf.case >= maf.contr, 1, -1); #set up in weightBurdenTest.R

ifelse(maf.case >= maf.contr, -1, 1);

}));

weights = weights\*DirectionWeight;

}

stat.perms[i] = do.call(testFunc, list(pheno.perm, genotypes, weights, caseN, controlN));

}

pValue = (sum(stat.perms >= stat)+0.5)/(length(stat.perms)+1);

return (list(geneSize=ncol(genotypes), permuN=i, pvalue=pValue));

}

}

#######################################################

#Description: Calculate the LRT statistic according to Bernoulli distribution. The burden score can be weighted. The LRT statistic is gotten at the best burden score threshold that maximizes the statistic.

#Depends on:

#Arguments: phenotypes: A vector of binary phenotype values. Require: 1s indicate cases; 0s indicate controls.

# genotypes: Genotype data frame (cols: variants; rows: sample subjects)

# weights: A one-col data frame vector of variant weights wrt to genotypes. Default: all 1s (no weighting).

# caseN: Number of cases;

# controlN: Number of controls.

#Details:

#Value: LRT statistic

#Note:

#Author(s): ZWu, DZhang

#References: Chen, ..., Karchin 2013 PloS Paper

#See Also:

#Example:

#######################################################

LRTStat <- function(phenotypes, genotypes, weights=data.frame(rep(1, ncol(genotypes))), caseN, controlN)

{

burdenScores = data.matrix(genotypes) %\*% data.matrix(weights);

thresholds = c(unique(burdenScores), max(burdenScores)+1);

stats = array(0, dim=c(length(thresholds )));

for (i in 1:length(thresholds ))

{

Ys = (burdenScores >= thresholds[i]);

Ycase = sum(Ys[phenotypes==1]);

Ycontrol = sum(Ys[phenotypes==0]);

stats[i] = LRTStat.condi(Ycase, Ycontrol, caseN, controlN);

}

finalStat = max(stats[!is.na(stats)]);

return(finalStat);

}

#######################################################

#Description: Calculate the LRT statistic, conditional on given numbers of 1's in cases and controls

#Depends on:

#Arguments: Ycase: number of 1's in cases

# Ycontrol: number of 1's in controls.

# caseN: Number of cases;

# controlN: Number of controls.

#Details:

#Value: LRT statistic

#Note:

#Author(s): ZWu, DZhang

#References: Chen, ..., Karchin 2013 PloS Paper

#See Also:

#Example:

#######################################################

LRTStat.condi <- function(Ycase, Ycontrol, caseN, controlN)

{

pA = (Ycase + 1)/(caseN + 2);

pU = (Ycontrol + 1)/(controlN + 2);

p = (Ycase+ Ycontrol + 2)/(caseN+ controlN+ 4);

if (pA >= pU)

{

statistic = Ycase\*log10(pA)+(caseN-Ycase)\*log10(1-pA)+Ycontrol\*log10(pU)+(controlN-Ycontrol)\*log10(1-pU)-(Ycase+Ycontrol)\*log10(p)-(caseN+controlN-Ycase-Ycontrol)\*log10(1-p);

}

else

{

statistic = Ycase\*log10(pU)+(caseN-Ycase)\*log10(1-pU)+Ycontrol\*log10(pA)+(controlN-Ycontrol)\*log10(1-pA)-(Ycase+Ycontrol)\*log10(p)-(caseN+controlN-Ycase-Ycontrol)\*log10(1-p);

}

return(statistic);

}

direct structure based prediction results of the effect of snv on PPI

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| P00533 | P00533 | EGFR | EGFR | A | I | 3b2u | line8504 | A | 497 | R->K | Preserving | 1 | Neutral | 0.97 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P00533 | **P00533** | **EGFR** | **EGFR** | **A** | **B** | **3njp** | **line8504** | **A** | 497 | R->K | Preserving | 0.54 | Detrimental | 0.63 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P00749 | **Q03405** | **PLAU** | **PLAUR** | **A** | **U** | **3bt2** | **line20283** | **U** | 198 | K->R | Preserving | 0.9 | Detrimental | 0.47 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P01040 | **P01040** | **CSTA** | **CSTA** | **A** | **B** | **1n9j** | **line5181** | **A** | 96 | T->M | Preserving | 0.79 | Neutral | 0.69 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P01040 | **P07711** | **CSTA** | **CTSL** | **D** | **A** | **3kse** | **line5181** | **D** | 96 | T->M | Preserving | 1 | Neutral | 0.99 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P01040 | **P07858** | **CSTA** | **CTSB** | **C** | **A** | **3k9m** | **line5181** | **C** | 96 | T->M | Preserving | 1 | Neutral | 0.76 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P01133 | **P00533** | **EGF** | **EGFR** | **B** | **A** | **1nql** | **line8504** | **A** | 497 | R->K | Preserving | 0.92 | Neutral | 0.83 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P01343 | **P08833** | **IGF1** | **IGFBP1** | **C** | **H** | **2dsq** | **line8410** | **H** | 228 | I->M | # |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P02730 | **P02730** | **SLC4A1** | **SLC4A1** | **P** | **Q** | **1hyn** | **line17387** | **P** | 56 | K->E | Preserving | 0.75 | Neutral | 0.6 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P06865 | **P07686** | **HEXA** | **HEXB** | **A** | **B** | **2gk1** | **line15611** | **A** | 436 | I->V | # |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P09467 | **P09467** | **FBP1** | **FBP1** | **A** | **B** | **3kbz** | **line10359** | **A** | 217 | R->K | # |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P20591 | **P20591** | **MX1** | **MX1** | **A** | **B** | **3ljb** | **line21431** | **A** | 379 | V->I | Preserving | 0.98 | Neutral | 0.97 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P20827 | **P29317** | **EFNA1** | **EPHA2** | **B** | **A** | **3mbw** | **line2347** | **B** | 159 | D->V | # |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P29474 | **P29474** | **NOS3** | **NOS3** | **A** | **B** | **1m9m** | **line9663** | **A** | 297 | D->E | Preserving | 0.86 | Neutral | 0.65 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P35228 | **P35228** | **NOS2** | **NOS2** | **A** | **B** | **1nsi** | **line16938** | **A** | 221 | R->W | Preserving | 0.85 | Detrimental | 0.48 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P36404 | **Q9Y2Y0** | **ARL2** | **ARL2BP** | **A** | **B** | **3doe** | **line12905** | **A** | 141 | V->A | Preserving | 0.86 | Neutral | 0.92 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Q9H2X3 | **Q9H2X3** | **CLEC4M** | **CLEC4M** | **A** | **B** | **1k9j** | **line19024** | **A** | 291 | D->N | Preserving | 0.99 | Neutral | 0.98 |

## Hypertension related gene list (based on literature)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ABCA3 | ACSM3 | ACVRL1 | ADC | ADD1 | ADRA1A |
| AGT | **AGTR1** | **ATP1B1** | **ATP2B1** | **BMPR2** | **CACNB2** |
| CAV1 | **CLCNKB** | **CPS1** | **CSK** | **CUL3** | **CYP11B1** |
| CYP11B2 | **CYP17A1** | **CYP3A5** | **ECE1** | **EDN3** | **FGF5** |
| GNAS | **GNB3** | **HFE** | **HSD11B2** | **KCNJ1** | **KCNK3** |
| KCNMB1 | **KLHL3** | **LSP1** | **MADH9** | **MECOM** | **MTHFR** |
| NOS2 | **NOS2A** | **NOS3** | **NPR3** | **NR3C2** | **PLEKHA7** |
| PNMT | **PTGIS** | **RET** | **RETN** | **RGS5** | **SARS2** |
| SCNN1B | **SCNN1G** | **SDHA** | **SDHAF2** | **SDHB** | **SDHC** |
| SDHD | **SELE** | **SH2B3** | **SLC12A1** | **SLC12A3** | **SMAD9** |
| SOX6 | **TBX3** | **TBX5** | **TNNT3** | **ULK4** | **UMOD** |
| VHL | **WNK1** | **WNK4** | **ZNF652** |  |  |

## Hypertension centered network- interactions from HINT databse

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| A2M | NOS3 |  |  | NOS3 | | EFEMP2 | |
| ACTB | **SMAD9** |  |  | **NOS3** | | **EXOC6** | |
| ACTN2 | **NOS3** |  |  | **NOS3** | | **FIS1** | |
| ACTN4 | **NOS3** |  |  | **NOS3** | | **HTRA1** | |
| ACVR1 | **BMPR2** |  |  | **NOS3** | | **IMMT** | |
| ACVR2A | **ACVRL1** |  |  | **NOS3** | | **KANK2** | |
| ACVRL1 | **ACVRL1** |  |  | **NOS3** | | **MAST1** | |
| ACVRL1 | **ENG** |  |  | **NOS3** | | **MPRIP** | |
| ACVRL1 | **TGFB1** |  |  | **NOS3** | | **NOS3** | |
| ACVRL1 | **TGFBR2** |  |  | **NOS3** | | **PI4K2A** | |
| ADD1 | **C1orf109** |  |  | **NOS3** | | **PPP2R5A** | |
| ADD1 | **CSNK2B** |  |  | **NOS3** | | **RNF31** | |
| ADD1 | **GEMIN7** |  |  | **NOS3** | | **RNF32** | |
| ADD1 | **HMG20A** |  |  | **NOS3** | | **ST13** | |
| ADD1 | **MAP1LC3B** |  |  | **NOS3** | | **TXNDC11** | |
| ADD1 | **PRKCD** |  |  | **NOS3** | | **UMPS** | |
| ADD1 | **SPTA1** |  |  | **NPPB** | | **NPR3** | |
| ADD1 | **TK1** |  |  | **NPPC** | | **NPR3** | |
| AES | **TBX3** |  |  | **NR3C2** | | **EIF3I** | |
| AGT | **ACE2** |  |  | **NR3C2** | | **NCOA1** | |
| AGT | **AGT** |  | **NR3C2** | | **PROX1** | |
| AGT | **CTSG** |  | **NR3C2** | | **PSMC5** | |
| AGT | **EWSR1** |  | **NR3C2** | | **SRC** | |
| AGT | **GRB2** |  | **NR3C2** | | **TRIM24** | |
| AGT | **PRCP** |  | **OAZ3** | | **ADC** | |
| AGTR1 | **PDE6H** |  | **PICK1** | | **PLEKHA7** | |
| AGT | **REN** |  | **PLCG1** | | **RET** | |
| AKT1 | **NOS3** |  | **PNMT** | | **KLHL8** | |
| AP2A1 | **SMAD9** |  | **PPL** | | **PLEKHA7** | |
| ARHGDIA | **SDHC** |  | **PRKAR1A** | | **WNK1** | |
| ARNT | **SMAD9** |  | **PRKAR1B** | | **WNK1** | |
| ATP1B1 | **ATP1B1** |  | **RANBP9** | | **WNK1** | |
| ATP1B1 | **CRIP2** |  | **RAP1GAP2** | | **WNK1** | |
| ATP1B1 | **DDAH2** |  | **RASAL3** | | **WNK1** | |
| ATP1B1 | **EZH2** |  | **RET** | | **FRS2** | |
| ATP1B1 | **LRIF1** |  | **RET** | | **NOTCH2NL** | |
| ATP1B1 | **MLL4** |  | **RET** | | **RET** | |
| ATP1B1 | **PSME1** |  | **RET** | | **SHC1** | |
| ATP1B1 | **RIF1** |  | **RET** | | **SHC3** | |
| ATP1B1 | **TRMT2A** |  | **RET** | | **STAT3** | |
| ATP2B1 | **ATP2B1** |  | **SCNN1B** | | **NEDD4L** | |
| ATP8 | **WNK1** |  | **SCNN1B** | | **STX1A** | |
| ATXN1 | **WNK1** |  | **SCNN1B** | | **WWP2** | |
| B2M | **HFE** |  | **SCNN1G** | | **NEDD4L** | |
| BAD | **WNK1** |  | **SCNN1G** | | **STX1A** | |
| BAG3 | **WNK1** |  | **SDHAF2** | | **CCDC90B** | |
| BDKRB2 | **NOS3** |  | **SDHAF2** | | **SDHAF2** | |
| BMP2 | **BMPR2** |  | **SDHB** | | **PIK3R5** | |
| BMP4 | **BMPR2** |  | **SDHB** | | **SPRY2** | |
| BMP7 | **BMPR2** |  | **SDHC** | | **SDHC** | |
| BMPR2 | **BMPR2** |  | **SELE** | | **SELPLG** | |
| BMPR2 | **GDF5** |  | **SH2B3** | | **SH2B3** | |
| BMPR2 | **MAPK8** |  | **SH2B3** | | **USP32P1** | |
| BMPR2 | **TGFBR1** |  | **SLC9A1** | | **WNK1** | |
| CA8 | **TBX3** |  | **SMAD2** | | **SMAD9** | |
| CACNB2 | **CTBP2** |  | **SMAD3** | | **SMAD9** | |
| CACNB2 | **HEXIM2** |  | **SMAD4** | | **SMAD9** | |
| CACNB2 | **PRKACA** |  | **SMAD9** | | **ABTB1** | |
| CAV1 | **CAV1** |  | **SMAD9** | | **AFF1** | |
| CAV1 | **CAV2** |  | **SMAD9** | | **ARHGAP9** | |
| CAV1 | **CSK** |  | **SMAD9** | | **ARID1B** | |
| CAV1 | **EGFR** |  | **SMAD9** | | **ASB2** | |
| CAV1 | **PLD1** |  | **SMAD9** | | **ASH2L** | |
| CAV1 | **PTPN1** |  | **SMAD9** | | **BAZ1A** | |
| CAV1 | **SRC** |  | **SMAD9** | | **C10ORF2** | |
| CAV1 | **TRAF2** |  | **SMAD9** | | **CAMSAP1** | |
| CBL | **RET** |  | **SMAD9** | | **CEP135** | |
| CCNDBP1 | **PLEKHA7** |  | **SMAD9** | | **CHPF** | |
| CENPJ | **WNK1** |  | **SMAD9** | | **CLPB** | |
| CHAF1A | **WNK1** |  | **SMAD9** | | **CPXM2** | |
| CLK2 | **ECE1** |  | **SMAD9** | | **CSRP3** | |
| CLTC | **WNK1** |  | **SMAD9** | | **CTR9** | |
| CNTN1 | **SCNN1B** |  | **SMAD9** | | **CXXC5** | |
| COPS5 | **WNK1** |  | **SMAD9** | | **DIAPH3** | |
| CSDE1 | **WNK1** |  | **SMAD9** | | **DKK1** | |
| CSH1 | **SMAD9** |  | **SMAD9** | | **DNAJA3** | |
| CSK | **DOK3** |  | **SMAD9** | | **DNAJC7** | |
| CSK | **FGR** |  | **SMAD9** | | **DSTN** | |
| CSK | **FYN** |  | **SMAD9** | | **EIF3C** | |
| CSK | **KANK4** |  | **SMAD9** | | **EIF3F** | |
| CSK | **LCK** |  | **SMAD9** | | **ERVV-1** | |
| CSK | **PTPRB** |  | **SMAD9** | | **EVC2** | |
| CSK | **PTPRC** |  | **SMAD9** | | **EXPH5** | |
| CSK | **PTPRG** |  | **SMAD9** | | **HEY1** | |
| CSK | **PTPRJ** |  | **SMAD9** | | **HEYL** | |
| CSK | **PTPRK** |  | **SMAD9** | | **HUWE1** | |
| CSK | **PTPRO** |  | **SMAD9** | | **KDM6A** | |
| CSK | **PTPRZ1** |  | **SMAD9** | | **KIAA0226** | |
| CSK | **PXN** |  | **SMAD9** | | **LEMD3** | |
| CSK | **RGS16** |  | **SMAD9** | | **LMO4** | |
| CSK | **SHC1** |  | **SMAD9** | | **MAN1A2** | |
| CSK | **SRC** |  | **SMAD9** | | **MAN1C1** | |
| CSNK2B | **SDHA** |  | **SMAD9** | | **MAN2B1** | |
| CTBP1 | **MECOM** |  | **SMAD9** | | **MBD1** | |
| CUL3 | **ABTB1** |  | **SMAD9** | | **MCM3AP** | |
| CUL3 | **ABTB2** |  | **SMAD9** | | **METAP1** | |
| CUL3 | **BTBD6** |  | **SMAD9** | | **MGAT1** | |
| CUL3 | **GMCL1** |  | **SMAD9** | | **MIA3** | |
| CUL3 | **KCTD13** |  | **SMAD9** | | **MLL2** | |
| CUL3 | **KCTD6** |  | **SMAD9** | | **MLL4** | |
| CUL3 | **KCTD7** |  | **SMAD9** | | **MOGAT1** | |
| CUL3 | **KCTD9** |  | **SMAD9** | | **MTCH1** | |
| CUL3 | **KEAP1** |  | **SMAD9** | | **MTMR10** | |
| CUL3 | **KLHL12** |  | **SMAD9** | | **MTMR11** | |
| CUL3 | **KLHL2** |  | **SMAD9** | | **NAGK** | |
| CUL3 | **KLHL3** |  | **SMAD9** | | **OTUB1** | |
| CUL3 | **ZMAT4** |  | **SMAD9** | | **PABPC4** | |
| CYP11A1 | **SMAD9** |  | **SMAD9** | | **PAPPA** | |
| DOK1 | **RET** |  | **SMAD9** | | **PELP1** | |
| DST | **SMAD9** |  | **SMAD9** | | **PHKA2** | |
| E4F1 | **SMAD9** |  | **SMAD9** | | **PIR** | |
| ECE1 | **EDN1** |  | **SMAD9** | | **PKP2** | |
| ECE1 | **KRTAP10-1** |  | **SMAD9** | | **PLEC** | |
| ECE1 | **KRTAP10-3** |  | **SMAD9** | | **PNPLA2** | |
| ECE1 | **KRTAP10-5** |  | **SMAD9** | | **PPARD** | |
| ECE1 | **KRTAP10-8** |  | **SMAD9** | | **PPP2R5E** | |
| ECE1 | **KRTAP1-3** |  | **SMAD9** | | **PSAP** | |
| EDN3 | **EDNRB** |  | **SMAD9** | | **PSMD8** | |
| EEF1A1 | **SDHAF2** |  | **SMAD9** | | **QARS** | |
| EFEMP1 | **NOS3** |  | **SMAD9** | | **RANBP9** | |
| EGFR | **SH2B3** |  | **SMAD9** | | **RFX1** | |
| EIF3A | **WNK1** |  | **SMAD9** | | **RMND5A** | |
| EIF3E | **SMAD9** |  | **SMAD9** | | **RNF123** | |
| ELAVL1 | **NOS3** |  | **SMAD9** | | **RRBP1** | |
| ELAVL3 | **NOS3** |  | **SMAD9** | | **SECISBP2** | |
| EPB41L2 | **WNK1** |  | **SMAD9** | | **SF3B1** | |
| EPRS | **SMAD9** |  | **SMAD9** | | **SIL1** | |
| ERBB2 | **SH2B3** |  | **SMAD9** | | **SMAD9** | |
| ERBB3 | **SH2B3** |  | **SMAD9** | | **SMAP1** | |
| ESR2 | **WNK4** |  | **SMAD9** | | **SMG1** | |
| EWSR1 | **KCNMB1** |  | **SMAD9** | | **SNRNP70** | |
| FAM13B | **WNK1** |  | **SMAD9** | | **SPTBN1** | |
| FAM53C | **WNK1** |  | **SMAD9** | | **STAG1** | |
| FAM65B | **WNK1** |  | **SMAD9** | | **SVEP1** | |
| FAM82A2 | **WNK1** |  | **SMAD9** | | **TBCD** | |
| FGF5 | **FGFR3** |  | **SMAD9** | | **TERF1** | |
| FHL3 | **WNK1** |  | **SMAD9** | | **TINAGL1** | |
| FHOD1 | **WNK1** |  | **SMAD9** | | **TMEM57** | |
| FLI1 | **SMAD9** |  | **SMAD9** | | **TRIM29** | |
| FLII | **SMAD9** |  | **SMAD9** | | **TRIP12** | |
| FLNA | **VHL** |  | **SMAD9** | | **TTC37** | |
| FLNC | **WNK1** |  | **SMAD9** | | **UBA6** | |
| FN1 | **SMAD9** |  | **SMAD9** | | **UBE3A** | |
| FN1 | **VHL** |  | **SMAD9** | | **UBQLN1** | |
| FTL | **SMAD9** |  | **SMAD9** | | **UBQLN4** | |
| GCDH | **NOS3** |  | **SMAD9** | | **UNC45A** | |
| GEM | **SDHB** |  | **SMAD9** | | **VCPIP1** | |
| GNB3 | **GNG3** |  | **SMAD9** | | **VPS8** | |
| GNB3 | **GNG4** |  | **SMAD9** | | **XAB2** | |
| GNB3 | **TGFBR1** |  | **SMAD9** | | **YWHAQ** | |
| GOLGA2 | **NOS3** |  | **SMAD9** | | **ZEB2** | |
| GPR25 | **RETN** |  | **SMAD9** | | **ZNF484** | |
| GRB2 | **SH2B3** |  | **SMAD9** | | **ZNF557** | |
| GRN | **SMAD9** |  | **SMAD9** | | **ZNF587** | |
| HDAC9 | **WNK1** |  | **SMAD9** | | **ZNF592** | |
| HFE | **HFE** |  | **SMAD9** | | **ZNF8** | |
| HFE | **TFR2** |  | **SMAD9** | | **ZNF83** | |
| HFE | **TFRC** |  | **SMAD9** | | **ZSCAN4** | |
| HIF1A | **VHL** |  | **SSFA2** | | **WNK1** | |
| HIVEP2 | **WNK1** |  | **STK39** | | **WNK4** | |
| HSP90AA1 | **NOS3** |  | **SYNPO** | | **WNK1** | |
| IKBKAP | **SDHAF2** |  | **TBX3** | | **PLEKHF2** | |
| IMMT | **SDHAF2** |  | **TBX3** | | **PRR20A** | |
| KCNK3 | **YWHAB** |  | **TBX3** | | **TOLLIP** | |
| KCNK3 | **YWHAZ** |  | **TBX5** | | **BAIAP2** | |
| KEAP1 | **KLHL3** |  | **TBX5** | | **ZMYND10** | |
| KIAA0232 | **WNK1** |  | **TCEB1** | | **VHL** | |
| KLHL3 | **C6orf165** |  | **TCEB2** | | **VHL** | |
| KLHL3 | **KLHL12** |  | **TNFAIP1** | | **CUL3** | |
| KLHL3 | **KLHL3** |  | **TNFAIP3** | | **WNK1** | |
| LCK | **SH2B3** |  | **TNNI2** | | **TNNT3** | |
| LCP2 | **WNK1** |  | **TNNT3** | | **HAP1** | |
| LNPEP | **SMAD9** |  | **TNNT3** | | **NUDT3** | |
| LRMP | **WNK1** |  | **TNNT3** | | **SNUPN** | |
| LRP5 | **SMAD9** |  | **TNNT3** | | **TNNT3** | |
| MADD | **WNK1** |  | **TNNT3** | | **TSG101** | |
| MAGEA11 | **NOS3** |  | **TNRC6A** | | **WNK1** | |
| MAP2K1 | **WNK1** |  | **TNS1** | | **WNK1** | |
| MDFI | **VHL** |  | **TSC22D1** | | **WNK1** | |
| MECOM | **MECOM** |  | **ULK4** | | **SMURF1** | |
| MECOM | **SMAD3** |  | **VBP1** | | **VHL** | |
| MEOX2 | **RGS5** |  | **VHL** | | **CUL2** | |
| MORF4L2 | **KLHL3** |  | **VHL** | | **ZNF512B** | |
| MPHOSPH9 | **WNK1** |  | **WNK1** | | **C5ORF25** | |
| MSL2 | **WNK1** |  | **WNK1** | | **CGNL1** | |
| MTHFR | **NAA38** |  | **WNK1** | | **SYNPO2** | |
| MYH9 | **WNK1** |  | **WNK1** | | **WNK1** | |
| NEDD4L | **WNK1** |  | **WNK1** | | **WNK2** | |
| NEDD4 | **SCNN1B** |  | **WWTR1** | | **WNK1** | |
| NEDD4 | **SCNN1G** |  | **XRCC6** | | **SDHC** | |
| NEDD8 | **CUL3** |  | **YWHAE** | | **WNK1** | |
| NOS2 | **NOS2** |  | **YWHAG** | | **WNK1** | |
| NOS2 | **RAC1** |  | **YWHAZ** | | **WNK1** | |
| NOS2 | **SLC9A3R1** |  | **ZFP106** | | **WNK1** | |
| NOS3 | **CDC37** |  | **ZNF839** | | **WNK1** | |
|  |  |  | **ZYX** | | **WNK1** | |

Structurally Resolved Hypertension Centered Network through INstruct database

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| BMP7 | BMPR2 |  | SMAD9 | | | SMAD9 | |
| NOS3 | **NOS3** |  | **FYN** | | | **CSK** | |
| SMAD3 | **SMAD9** |  | **ACVRL1** | | | **ACVRL1** | |
| SH2B3 | **ERBB3** |  | **RET** | | | **SHC1** | |
| ACVRL1 | **ACVR2A** |  | **SMAD9** | | | **SMAD9** | |
| SMAD9 | **SMAD2** |  | **SRC** | | | **CSK** | |
| ACVRL1 | **ACVR2A** |  | **SRC** | | | **CSK** | |
| SMAD9 | **SMAD4** |  | **SH2B3** | | | **SH2B3** | |
| SMAD9 | **SMAD2** |  | **GNB3** | | | **GNG3** | |
| PTPRB | **CSK** |  | **ACVRL1** | | | **ACVRL1** | |
| NOS2 | **NOS2** |  | **STAT3** | | | **RET** | |
| TFR2 | **HFE** |  | **SHC1** | | | **CSK** | |
| SRC | **CSK** |  | **PLCG1** | | | **RET** | |
| PTPRC | **CSK** |  | **RET** | | | **RET** | |
| FGR | **CSK** |  | **PTPRJ** | | | **CSK** | |
| NOS3 | **NOS3** |  | **PTPRB** | | | **CSK** | |
| CSK | **PTPRO** |  | **TGFBR1** | | | **BMPR2** | |
| ACVR1 | **BMPR2** |  | **MECOM** | | | **MECOM** | |
| CSK | **PXN** |  | **SRC** | | | **CSK** | |
| HFE | **HFE** |  | **FYN** | | | **CSK** | |
| ATP2B1 | **ATP2B1** |  | **LCK** | | | **CSK** | |
| TCEB1 | **VHL** |  | **WNK1** | | | **WNK1** | |
| SH2B3 | **GRB2** |  | **PTPRB** | | | **CSK** | |
| NPPC | **NPR3** |  | **ATP2B1** | | | **ATP2B1** | |
| PTPRC | **CSK** |  | **TGFBR1** | | | **BMPR2** | |
| FGF5 | **FGFR3** |  | **SRC** | | | **CSK** | |
| SDHAF2 | **SDHAF2** |  | **NOS2** | | | **NOS2** | |
| BMP4 | **BMPR2** |  | **HFE** | | | **HFE** | |
| ACVR1 | **BMPR2** |  | | **NEDD8** | **CUL3** | |
| NOS3 | **NOS3** |  | | **NOS2** | **NOS2** | |
| AGT | **CTSG** |  | | **PTPRJ** | **CSK** | |
| FGR | **CSK** |  | | **PTPRJ** | **CSK** | |
| GNB3 | **GNG4** |  | | **PTPRG** | **CSK** | |
| RET | **RET** |  | | **PTPRG** | **CSK** | |
| SRC | **CSK** |  | | **TGFBR1** | **BMPR2** | |
| HFE | **HFE** |  | | **FGR** | **CSK** | |
| NOS2 | **NOS2** |  | | **NOS2** | **NOS2** | |
| FGR | **CSK** |  | | **SRC** | **CSK** | |
| AGT | **AGT** |  | | **PTPRZ1** | **CSK** | |
| WNK1 | **PRKAR1B** |  | | **FGR** | **CSK** | |
| PTPRZ1 | **CSK** |  | | **LCK** | **CSK** | |
| FYN | **CSK** |  | | **SH2B3** | **GRB2** | |
| ATP1B1 | **ATP1B1** |  | | **LCK** | **CSK** | |
| FYN | **CSK** |  | | **ATP2B1** | **ATP2B1** | |
| EGFR | **SH2B3** |  | | **SH2B3** | **SH2B3** | |
| BMP2 | **BMPR2** |  | | **NOS3** | **NOS3** | |
| LCK | **CSK** |  | | **PTPRZ1** | **CSK** | |
| FGR | **CSK** |  | | **SHC1** | **CSK** | |
| HFE | **TFRC** |  | | **SRC** | **CSK** | |
| FYN | **CSK** |  | | **FGR** | **CSK** | |
| FYN | **CSK** |  | | **PTPRC** | **CSK** | |
| SRC | **CSK** |  | | **FYN** | **CSK** | |
| NOS3 | **NOS3** |  | | **WNK1** | **MAP2K1** | |
| LCK | **CSK** |  | | **BMPR2** | **BMPR2** | |
| NOS3 | **NOS3** |  | | **FYN** | **CSK** | |
| B2M | **HFE** |  | | **SDHC** | **SDHC** | |
| SH2B3 | **LCK** |  | | **ERBB2** | **SH2B3** | |
| SH2B3 | **SH2B3** |  | | **CSK** | **PTPRO** | |
| ACVRL1 | **ACVRL1** |  | | **ATP2B1** | **ATP2B1** | |
| PTPRG | **CSK** |  | | **FGF5** | **FGFR3** | |
| ATP2B1 | **ATP2B1** |  | | **ATP2B1** | **ATP2B1** | |
| TNNI2 | **TNNT3** |  | | **SHC1** | **CSK** | |
| ATP2B1 | **ATP2B1** |  | | **ATP2B1** | **ATP2B1** | |
| ACVRL1 | **ACVR2A** |  | | **NOS2** | **NOS2** | |
| NOS2 | **NOS2** |  | | **LCK** | **CSK** | |
| B2M | **HFE** |  | | **VHL** | **HIF1A** | |
| ACVR1 | **BMPR2** |  | | **AGT** | **REN** | |
| SH2B3 | **LCK** |  | | **NOS3** | **NOS3** | |
| WNK1 | **PRKAR1A** |  | | **WNK1** | **WNK2** | |
| FGR | **CSK** |  | | **WNK4** | **STK39** | |
| LCK | **CSK** |  | | **FGR** | **CSK** | |
| LCK | **CSK** |  | | **SMAD9** | **SMAD4** | |
| CSK | **PTPRO** |  | | **MAPK8** | **BMPR2** | |
| SH2B3 | **LCK** |  | | **SMAD3** | **SMAD9** | |
| PLCG1 | **RET** |  | | **BMPR2** | **BMPR2** | |
| LCK | **CSK** |  | | **SH2B3** | **LCK** | |
| TNNT3 | **TNNT3** |  | | **SH2B3** | **GRB2** | |
| SHC3 | **RET** |  | | **FYN** | **CSK** | |
|  |  |  | | **NPPB** | **NPR3** | |