A framework to decipher the genetic architecture of combinations of complex diseases: applications in cardiovascular medicine

Liangying Yin¹, Carlos Kwan-long Chau¹, Yu-Ping Lin¹, Hon-Cheong So¹-5*

¹School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong
²KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research of Common Diseases, Kunming Institute of Zoology and The Chinese University of Hong Kong, China
³Department of Psychiatry, The Chinese University of Hong Kong, Hong Kong
⁴CUHK Shenzhen Research Institute, Shenzhen, China
⁵Margaret K.L. Cheung Research Centre for Management of Parkinsonism, The Chinese University of Hong Kong, Shatin, Hong Kong

Correspondence to: Hon-Cheong So, Lo Kwee-Seong Integrated Biomedical Sciences Building, The Chinese University of Hong Kong, Shatin, Hong Kong. Tel: +852 3943 9255; E-mail: hcso@cuhk.edu.hk

Submitted to arxiv on 19 Mar 2020

Abstract

Genome-wide association studies (GWAS) have proven to be a highly useful design in revealing the genetic basis of complex diseases, for example in cardiovascular medicine. At present, most of the GWAS are studies of a particular single disease diagnosis (e.g., coronary heart disease, asthma etc.) against controls. However, in clinical practice, an individual often has more than one condition/disorder. For example, patients with coronary artery disease (CAD) are often comorbid with diabetes mellitus (DM). Along a similar line, it is often clinically meaningful to study patients with one disease but without a comorbid condition. For example, patients with both DM and obesity may have different pathophysiology from those with DM but normal weight. From another angle, there may exist different ‘subtypes’ of a disease, as characterized by the absence or presence of comorbid conditions or disorders.

In this study, we developed a statistical framework to uncover susceptibility variants for comorbid disorders (or a disorder without comorbidity), which requires GWAS summary statistics only. In essence, we are mimicking a case-control GWAS in which the cases are affected with comorbidities or a particular disease but without a relevant comorbid condition (in either case, we may consider the cases as those affected by a specific ‘subtype’ of disease as characterized by the presence or absence of comorbid conditions). We also extended our methodology to deal with continuous traits with clinically meaningful categories (e.g. lipid
levels). In addition, we illustrated how the analytic framework may be extended to more than two traits/disorders. We verified the feasibility and validity of our method by applying it to simulated scenarios and four cardiometabolic traits.

Our proposed framework performed well in revealing susceptibility variants for comorbidities as well as only single trait without comorbid conditions in all simulated scenarios. Application to cardiometabolic traits further verified the validity of our method in unlocking the underlying susceptibility loci for cardiometabolic diseases ‘subtypes’. We also analyzed the genes, pathways, cell-types/tissues involved in these disease subtypes. LD-score regression analysis revealed some of these ‘subtypes’ may indeed be biologically distinct with low genetic correlation. Further Mendelian randomization analysis also found different causal effects of different subtypes to relevant complications. Taken together, the proposed methodology is useful in uncovering the genetic basis of disease ‘subtypes’, as characterized by the presence or absence of comorbidities. The findings are of both scientific and clinical value, and the proposed method may open a new avenue to analyzing GWAS data.
Introduction

Genome-wide association studies have proven to be a highly useful design in revealing the genetic basis of many complex diseases, and has contributed to the understanding of the mechanisms of many diseases, for example in cardiovascular medicine and psychiatry\(^1,2\). GWAS data also have the potential to be directly translated to clinical practice, for example in risk prediction by polygenic scores, disease subtyping and drug discovery\(^3-8\).

To date, more than 4000 GWAS have been conducted to date (https://www.ebi.ac.uk/gwas/), and the emergence of large biobanks (such as the UK Biobank) has further boosted the variety and amount of genomic data available. Most of the GWAS (or human sequencing studies) are studies of a particular single disease (e.g. schizophrenia, coronary heart disease, asthma etc.) against controls. However, in clinical practice, an individual patient often has more than one condition/disorder. For example, patients with coronary artery disease (CAD) are often comorbid with diabetes mellitus (DM), while DM patients often have obesity; in psychiatry, patients with schizophrenia have a higher probability of having comorbid substance abuse, depression, obsessive compulsive disorder and many other psychiatric disorders\(^9\).

Patients with both DM and CAD, for example, might share different pathophysiology than patients with DM alone or CAD alone. Viewed in another way, patients with both DM and CAD may be considered a ‘distinct’ entity, and its etiology and genetic basis may warrant further investigations. Ideally, we would perform a GWAS with ‘cases’ defined as patients having both disorders, and compared their genotypes with control subjects. However, recruiting patients with both disorders is usually more costly than recruiting those with a single disorder.

Along a similar line, it is often clinically meaningful to study patients with one disease but without a comorbid condition. For example, more than 90% of DM patients are overweight\(^10\); however there are still DM subjects with normal weight, who may represent a specific subtype of DM. In the ideal case, we will wish to recruit patients with DM but normal weight as ‘cases’ and compare them against controls. However, the complexity and cost of recruitment is then increased (compared to the standard design of DM vs controls), hence limiting the practicality of such studies. As another example, it was estimated that ~75% of patients with depression also suffer from anxiety disorders\(^11\); however, the rest of the patients having depression but no anxiety disorders may represent a specific ‘subtype’ of depression. By studying the genetic basis of such subgroup of patients, we will be able gain deeper understanding into the heterogeneity and pathophysiology of depression.
As raised in the above examples, many diseases are highly heterogeneous. Patients with the same diagnosis may have different clinical presentations and prognosis, and share different etiologies. Through stratifying patients of the same diagnosis by the presence or absence of comorbid condition(s) and uncovering the genetic basis for each subgroup, we may gain better insight into the pathophysiology of the disorder. This will ultimately lead to more targeted interventions or prevention strategies for distinct subgroups of patients with the same diagnosis.

The aim of this study is to develop a framework to decipher the genetic architecture of multiple diseases in combination. Specifically, we wish to uncover susceptibility variants for comorbid conditions, and for ‘subtype’ of a disease without comorbid condition(s) (e.g. DM without obesity/overweight, depression without anxiety, CAD without hyperlipidemia etc.). The framework can be potentially applied to any complex diseases, and only summary statistics are required, which greatly extends the applicability of the methodology. In essence, we are ‘mimicking’ a case-control GWAS in which cases are affected with comorbidities, or affected by a particular disease but without a relevant comorbid condition (in either case, we may consider cases as having a ‘subtype’ of the disease as characterized by the presence or absence of comorbidities).

We will then apply such methods to cardiovascular disorders (CVD), uncover genes and pathways associated, and find out casual clinical risk factors for comorbid diseases (or disease without comorbidity). This study is mainly focused on applications in cardiovascular medicine in view of its high public health importance and that many CVD are related to each other; nevertheless, the method itself is widely applicable to any complex traits. The presented computational framework can be considered an extension of the method by Nieuwboer et al., for which the main focus was on finding susceptibility variants for functions of quantitative phenotypes such as body mass index (= weight/height²). Here we modified and further developed the approach to accommodate binary outcomes, which are more commonly studied in GWAS, and proposed new applications in deciphering the genetic basis of comorbid disorders and disease subtypes as characterized by the presence (or absence) of a related trait. In addition, we also developed new methods to handle clinically defined categories of quantitative traits, approaches to compute covariance between phenotypes (which are required as input) as well as more general extensions to more than two diseases/traits.

Here we highlight a few related directions of research. One related research area is the finding of genetic correlation between complex traits. LD score regression (LDSR) is a commonly used technique to compute genetic correlation between traits, although there are also limitations to this approach, for example there are inherent assumptions that may not be fully fulfilled in practice. For example it assumes that distribution of causal variants in the genome is independent of the LD structure, and that (ideally) the variance explained by each SNP is the same. Also, if the SNP effect sizes are not normally distributed, the procedure may become
less efficient, resulting in lower power to detect true associations\textsuperscript{14}. Another related approach is to construct polygenic risk scores (PRS) for the first trait, and then test associations with the second. This approach however cannot easily accommodate sample overlap. There is a fundamental difference between LDSR or PRS with the approach presented here. LDSR/PRS aims to discover \textit{overall} genetic correlation or overlap between disorders, but are \textit{not designed for finding specific susceptibility variants} underlying comorbid disorders or a disorder without comorbid condition(s).

Another intuitive approach is to find variants passing a significance threshold (e.g. $p<5\text{e}-8$) for each trait, and directly find the overlapping variants. However, the setting of the significance threshold could be arbitrary, as setting a very stringent threshold (such as the conventional genome-wide significance cut-off) will lead to low power and carried the risk of missing genuine genetic variants contributing to comorbidity; setting a relaxed threshold (e.g. $p<0.05$) will result in increased false positive rates. Another approach to develop more formal statistical procedures to find shared genetic loci. For example, a co-localization approach based on summary statistics was proposed in Giambartolomei’s paper\textsuperscript{15}. The approach is Bayesian and outputs posterior probabilities that the variant is a genuine association signal for \textit{both} traits. A limitation is that prior probabilities for different configurations of associations need to be specified, which may not be straightforward; difficulties may also arise for multiple independent associations at one locus. However, there are also \textit{fundamental differences} between the ‘co-localization’ approach and our methodology. Our methodology can be conceptualized as mimicking the GWAS of a case-control study in which the cases are affected with comorbid disorders (or disease without a comorbid trait); as such, we are able to derive \textit{effect sizes} [e.g. odds ratios(OR)] of individual genetic variants. We may conclude, for example, the allele A (compared to a) of a certain SNP confers an OR of 2.0 to comorbid depression and anxiety. On the other hand, we cannot derive effect sizes with the co-localization approach. Also, the presented method is based on the frequentist approach with p-values as measures of significance, which may appear more familiar to many biologists and clinicians. Since most GWAS analytic tools are developed based on frequentist methods or use p-values as input, it might be easier to perform secondary analysis (such as gene- and pathway-based analysis) with our methodology.

GWAS meta-analysis is another related topic. However, the principle of meta-analysis is different from the proposed approach which address genetic basis of \textit{combinations} of diseases/traits. In a meta-analysis, we aggregate evidence from different studies of the same or highly related traits to improve power; if one variant is highly significant in one large study, the final meta-analysis result will likely still be significant, regardless of the results of other studies. In addition, meta-analyses are not designed for finding genetic variants for a disease without a comorbid condition, such as DM with no overweight/obesity\textsuperscript{16}. 
Method

In this study we introduce a statistical framework which has the potential to uncover susceptibility loci for comorbid disorders (or a disorder without comorbidity). It allows one to approximate the GWAS statistics for a comorbidity or a single disorder without comorbid trait based on the GWAS summary statistics of corresponding disorders only.

Here, we start by providing the derivation of GWAS summary statistics of interested trait (either a comorbid disorder or only single disorder without comorbidity) based on individual summary statistics. Suppose $P_1$ and $P_2$ are two different binary clinical traits. The interested clinical trait $T = f(P_1, P_2)$ can be defined as a function of the corresponding phenotypes. Let $S \sim \text{bin}(n = 2, q)$ be a binomially distributed variable corresponding to the number of effect alleles (EA) of a biallelic SNP, where $q$ denotes the effect allele frequency. Suppose we have a multivariate linear regression model on a data set of size $N$, then we have:

$$
\begin{pmatrix}
P_{11} & P_{12} \\
P_{12} & P_{22}
\end{pmatrix} =
\begin{pmatrix}
1 & S_1 \\
1 & S_2
\end{pmatrix}
\begin{bmatrix}
\beta_{11} & \beta_{12} \\
\beta_{12} & \beta_{22}
\end{bmatrix} + \epsilon
$$

where $P$ is a $N \times 2$ matrix, $S$ is a $N \times 2$ matrix, $\beta$ is a $2 \times 2$ matrix, and $\epsilon$ is a $N \times 2$ matrix. We assume each row $\epsilon$ is independent and follows a normal distribution $\epsilon \sim N(0, \Sigma)$. If we have known the estimate for the matrix $\hat{\beta}$ (denoted by $\hat{\beta}$), standard errors of each $\hat{\beta}_{ij}$, the covariance matrix between $P_1$ and $P_2$, as well as the mean of these two phenotypes. This is equivalent to have the GWAS summary statistics of each phenotype and their phenotypic covariances. Our goal is to estimate $\gamma_0, \gamma_1$ for the target trait (either a comorbid disorder or only single disorder without comorbidity), i.e.,

$$
f(P_1, P_2) = T = \gamma_0 + \gamma_1S_i + \epsilon_i
$$

where $\epsilon_i$ follows a normal distribution with zero mean. Obviously, it’s equivalent to perform a GWAS for trait $T$. To realize this, we use the second-order Taylor approximation of $T$ around the point $\epsilon(s)$ for $s = 0, 1, 2$ where $\epsilon(s) := (E[P_{1i}|S_i = s], [P_{2i}|S_i = s])$. Here point $\epsilon(s)$ corresponds to the mean of the phenotypes of the individuals who has $s$ effect alleles on this SNP. The second-order Taylor approximation for the trait can be expressed as:

$$
L_i := f(\epsilon(s)) + \sum_{k=1}^{2} \frac{\partial f(\epsilon(s))}{\partial P_k} (P_{ki} - E[P_{ki}|S_i = s]) + \frac{1}{2} \sum_{i=1}^{2} \sum_{k=1}^{2} \frac{\partial^2 f(\epsilon(s))}{\partial P_i \partial P_k} (P_{ki} - E[P_{ki}|S_i = s])(P_{ki} - E[P_{ki}|S_i = s])
$$

where $\partial f(\epsilon(s))/\partial P_k$ and $\partial^2 f(\epsilon(s))/\partial P_l \partial P_k$ denote the first- and second-order partial derivates of $f$ with respect to the corresponding phenotypes respectively, calculated at point $\epsilon(s)$. Based on the linearity of the expectation operation, we have

$$
E[L_i|S_i = s] = E[f(\epsilon(s))|S_i = s] + \frac{1}{2} \sum_{i=1}^{2} \sum_{k=1}^{2} \frac{\partial^2 f(\epsilon(s))}{\partial P_i \partial P_k} (P_{ki} - E[P_{ki}|S_i = s])(P_{ki} - E[P_{ki}|S_i = s])|S_i = s
$$

where

$$
f(\epsilon(s)) = \frac{1}{2}f'(\epsilon(s)) + \epsilon
$$

6
Notably, the linearization is possible only if it meets certain conditions on the phenotype value space. To be more specific, division by 0 is not allowed. We can avoid this situation by linear transformation of the observed phenotypes and parameters in the $\beta$ matrix. If we neglect the minor errors incurred during the linearization process, we will have

$$E[L_i|S_i = s] = E[C_i|S_i = s] = \gamma_0 + \gamma_s s$$

Then we will have a direct approximation for $\gamma_0$ when $s = 0$, i.e.,

$$\gamma_0 = f(\beta_{s01}, \beta_{s02}) + \frac{1}{2} f(\beta_{s01}, \beta_{s02})$$

We can also estimate $\gamma_1$ by evaluate it for $s = 1, 2$ and weighting the results by their relative population frequency, i.e.,

$$\gamma_1 = \frac{2q(1-q)^2}{2q(1-q)^4} \left( f(\beta_{s11} + \beta_{s01}, \beta_{s02} + \beta_{s12}) + \frac{1}{2} f(\beta_{s11} + \beta_{s01} + \beta_{s12}, \beta_{s02}) - \gamma_0 \right)$$

$$+ \frac{q^2}{2q(1-q)^4} \left( f(\beta_{s11} + 2\beta_{s12}, \beta_{s02} + 2\beta_{s12}) + 2 \left( f(\beta_{s11} + \beta_{s12}, \beta_{s02} + 2\beta_{s12}) - \gamma_0 \right) \right)$$

Since we don’t have the covariance matrix of $\hat{\beta}$, we need to estimate it between each of the $\hat{\beta}_{ik}$. Based on our multivariate linear regression assumption, the corresponding covariance matrix of $\hat{\beta}$ can be given by:

$$\text{Var}(\hat{\beta}) = (S^T)^{-1} \sum \Sigma$$

where $\Sigma$ is a $2 \times 2$ matrix with $\sum_{ik} = \text{Cov}(\epsilon_i, \epsilon_k)$, which is the covariance between errors in the linear regression of phenotypes $P_i, P_k$ on SNP S. To get the covariance matrix, one needs to analyze all the phenotypes at the same time which is not desirable. Based on the suggestions given by the original authors, we can get an approximation of $\text{Var}(\hat{\beta})$ instead of analyzing all phenotypes. We assume each individual SNP has small effect on the corresponding phenotype, so $\text{Var}(\epsilon_i) \approx \text{Var}(P_i)$ and $\text{Cov}(\epsilon_i, \epsilon_k) \approx \text{Cov}(P_i, P_k)$. Thus, we can infer

$$\text{Cov}(\hat{\beta}_{i1}, \hat{\beta}_{ik}) \approx \text{SE}_{i1} \text{Cor}(P_i, P_k) \text{SE}_{ik}$$

Notably, the above equation only holds for completely overlapped phenotypes. If there is only partial overlap, $\text{Cov}(\hat{\beta}_{i1}, \hat{\beta}_{1k})$ can be approximated as:

$$\text{Cov}(\hat{\beta}_{i1}, \hat{\beta}_{1k}) = \text{SE}_{i1} \text{Cor}(P_i, P_k) \frac{N_{ik}}{\sqrt{N_i N_k}} \text{SE}_{ik}$$

2.10

Where $N_i$ and $N_k$ are the number of individuals for the GWAS of $P_i$ and $P_k$ respectively, while $N_{ik}$ is the number of individuals both in the GWAS of $P_i$ and GWAS of $P_k$. If $\text{Cor}(P_i, P_k)$ can’t be directly calculated, we can use LD score regression to estimate $\text{Cor}(P_i, P_k) \frac{N_{ik}}{\sqrt{N_i N_k}}$ based on GWAS summary statistics. Notably, if there is no sample overlap between the phenotypes, then $N_{ik}$ is zero, so is the term $\text{Cov}(\hat{\beta}_{i1}, \hat{\beta}_{1k})$. The covariance between intercepts $\text{Cov}(\hat{\beta}_{0i}, \hat{\beta}_{0k})$ can be expressed as:

$$\text{Cov}(\hat{\beta}_{0i}, \hat{\beta}_{0k}) = \text{Cov}(\bar{P}_i - S\hat{\beta}_{1i}, \bar{F}_k - S\hat{\beta}_{1k}) = \text{Cov}(\bar{P}_i, \bar{P}_k) - S \text{Cov}(\hat{\beta}_{1i}, \bar{P}_k) - S \text{Cov}(\hat{\beta}_{1k}, \bar{P}_i) + S^2 \text{Cov}(\hat{\beta}_{1i}, \hat{\beta}_{1k})$$

2.11

Since $\text{Cov}(\hat{\beta}_{1i}, \bar{P}_i)$ is zero and $\text{Cov}(\bar{P}_i, \bar{P}_k)$ is negligible when sample size is large (typical GWAS sample sizes are $>10,000$ or more), the above equation can be simplified as:
Similarly, we get
\[ \text{Cov}(\beta_{i0}, \beta_{i1}) = \text{Cov}(\beta_{i1}, \beta_{i0}) = \check{S} \text{Cov}(\beta_{i1}, \beta_{i1}) \]
\[ \text{Cov}(\beta_{i1}, \beta_{i1}) = \text{Cov}(\beta_{i1}, \beta_{i0}) = \check{S} \text{Cov}(\beta_{i1}, \beta_{i1}) \]

A framework for application to binary phenotypes - uncovering the genetic basis of comorbid disorders or a single disorder without related comorbidity

The above derivations are based on continuous phenotypes. However, we are interested in disease traits which are usually binary. In this regard, we need to develop a framework to deal with binary phenotypes.

Conversion of coefficient (from logistic model to that under a linear model and vice versa)

In the above derivations it is assumed that we are dealing with coefficients obtained under a linear model. However, summary statistics for binary traits are usually derived from logistic regression. We therefore need to convert the coefficients from logistic models to those derived under linear models.

Lloyd-Jones et al\(^1\) proposed a method for transforming summary statistics based on linear regression to odds ratio (\(OR\)):

\[ OR_1 = \frac{k + \beta_1(1 - p)[1 - k + \beta_1 p]}{k - \beta_1 p[1 - k - \beta_1(1 - p)]} \]

where \(p\) indicates the effect allele frequency of the SNP under study \(S\), \(k\) represents the proportion of cases and \(\beta_1\) represents the coefficient under a linear model. This formula is useful for converting coefficients from a linear model to those under a logistic model and vice versa (see below). Note that the odds ratio \([=\exp(\beta)]\) estimate from a logistic regression is unbiased regardless of a retrospective or prospective design, with any level of over- or under-sampling of cases. This property however does not apply for linear regression\(^1\). To ensure that the final effect size estimate is close to the actual estimate when a prospective study is performed, we shall use the population lifetime risk estimate for \(k\). Intuitively the analysis is performed as if we were doing a prospective study in the population. In the final step we will convert the coefficient from a linear model back to a logistic coefficient, which we shall employ the lifetime time probability of the comorbidity \([i.e. \text{Pr}(P_1 \text{ and } P_2)]\) as input for \(k\).

As explained above, here we are also interested in the reverse of 2.14, i.e. solving for \(\beta_1\) (coefficient under a linear model) given the odds ratio (OR). Denoting the odds ratio (OR) of SNP \(S\) regressed on the binary phenotype by \(\alpha\), we have

\( (ak - k)(1 - k) = \beta[p(1 - p) + ap(1 - p)] + \beta_1[ak(1 - p) + ap - apk + kp + (1 - p)(1 - k)] \)

We could solve the above quadratic equation for \(\beta_1\). We choose the solution whose absolute value is smaller than the coefficient under a logistic model, i.e., \(abs(\beta_1) < abs(\log(\alpha))\). This choice could be verified by experimenting with different \(k\) and randomly generated \(p\) from uniform distribution with value range of [0.05,0.95]. As demonstrated by Fig.S1, the absolute values of coefficient (\(\beta\)) for binary traits derived from a linear regression is smaller than the coefficient(\(\alpha\)) under a logistic model, i.e., \(abs(\beta) < abs(\log(\alpha))\).
After the conversion, we may compute \( \tilde{y}_0 \) and \( \tilde{y}_1 \). We can employ the delta method\(^{19} \) to calculate the standard errors of \( \tilde{y}_0 \) and \( \tilde{y}_1 \). In essence, the delta method can be used to quantify variance of a function based on its first-order Taylor approximation. Suppose \( G(X) \) and \( U \) are the transform functions and mean vector of random variables \( X = (x_1, x_2, ..., x_n) \). The first-order Taylor expansion approximation for the function can be written as:

\[
G(X) = G(U) + \nabla G(U) \cdot (X - U) \tag{2.16}
\]

where \( \nabla G(U) \) is the gradient of \( G(X) \). Then, we can take the variance of this approximation as the estimation for the variance of \( G(X) \), i.e.,

\[
\text{Var}(G(X)) \approx \nabla G(U) \cdot \text{Cov}(X) \cdot \nabla G(X) \tag{2.17}
\]

Covariance between the coefficients can be derived based on the methods described above.

**Modeling comorbid disorders or a disorder without comorbidity**

Let \( P_1 \) and \( P_2 \) be two different binary clinical traits (coded as 0 and 1 for the absence and presence of disease respectively), the presence of comorbidity (Comor) can be expressed as:

\[
\text{Comor} = f(P_1, P_2) = P_1 \times P_2 \tag{2.18}
\]

Thus we can infer the corresponding coefficient estimates as follows:

\[
\text{Comor}(\hat{y}_1) = \beta_{\hat{y}_1} \times \beta_{\hat{y}_2} + \text{Cov}(P_1, P_2) \tag{2.19}
\]

\[
\text{Comor}(\hat{y}_1) = \frac{2q(1-q)}{2q(1-q) + q^2} \left[ \left( \beta_{\hat{y}_1} + \beta_{\hat{y}_2} \right) \times \left( \beta_{\hat{y}_1} + \beta_{\hat{y}_2} \right) + \text{Cov}(P_1, P_2) \right] - \text{Comor}(\hat{y}_0) \tag{2.20}
\]

Similarly, having a disorder \( (P_1) \) but without a specific comorbidity \( (P_2) \) (e.g. CAD without DM) can be expressed as:

\[
\text{Single} = f(P_1, P_2) = P_1 \times (1 - P_2) \tag{2.21}
\]

And the corresponding coefficient estimates can be estimated by:

\[
\text{Single}(\hat{y}_1) = \beta_{\hat{y}_1} \times (1 - \beta_{\hat{y}_2}) - \text{Cov}(P_1, P_2) \tag{2.22}
\]

\[
\text{Single}(\hat{y}_1) = \frac{2q(1-q)}{2q(1-q) + q^2} \left[ \left( \beta_{\hat{y}_1} + \beta_{\hat{y}_2} \right) \times (1 - \beta_{\hat{y}_2} - \beta_{\hat{y}_1}) - \text{Cov}(P_1, P_2) \right] - \text{Single}(\hat{y}_0) \tag{2.23}
\]

\[
+ \frac{q^2}{2q(1-q) + q^2} \left[ \left( \beta_{\hat{y}_1} + 2\beta_{\hat{y}_2} \right) \times (1 - \beta_{\hat{y}_2} - 2\beta_{\hat{y}_1}) - \text{Cov}(P_1, P_2) \right] - \text{Single}(\hat{y}_0) \tag{2.23}
\]

Note that \( \text{cov}(P_1, P_2) \) will cancel out in 2.20 and 2.23 hence this quantity does not affect the results.

**Extension to more than two traits**

The above proposed framework could also be extended to more than two traits. The only difference is that one of inputs should be the summary statistics of the comorbidity instead of a single disease, which could be derived from our proposed framework. For example, if we are interested in the genetic architecture of CAD
comorbid with T2DM and obesity, we could estimate the summary statistics of *CAD comorbid with T2DM* first. Given the results, we could re-apply our methodology again to deal with comorbidity with the 3rd trait (obesity). It is often difficult to extract the lifetime risk of more than 2 comorbid disorders from the literature. If this is the case, we could employ Mendelian randomization (MR) to infer the OR of comorbid 1st + 2nd trait on the third one first, then the overall lifetime risk can be computed based on the methodology described in a section below. In a similar vein, the proposed framework can be applied to an arbitrary number of traits by *sequential* application of our method.

**Application to clinically defined categories of quantitative traits**

It also worth noting that our proposed framework is applicable to clinically defined categories of quantitative traits. For example, hyperlipidemia is a risk factor for many cardiovascular diseases and clinical thresholds for LDL-C, HDL-C and triglycerides have been defined to facilitate the identification and treatment for subjects at high risks. However, GWAS summary data are only available for lipids as a quantitative trait. One may wish to identify genetic variants contributing to for example comorbid CAD and hyperlipidemia, which is clinically important. To realize this, we need to transform coefficients derived from linear regression (with outcome as a continuous trait) \( \beta_1 \) to coefficients from logistic regression (with outcome as a binary trait, such as hyperlipidemia or not) \( \beta_1^b \).

Suppose \( S \sim bin(n = 2, q) \) is a binomially distributed SNP (where \( q \) denotes the effect allele frequency,). For each continuous trait \( y \), we may model the effects of each SNP by:

\[
y = \beta_0 + \beta_1 S + \varepsilon \tag{2.29}
\]

where \( \varepsilon \) is an error term that follows a normal distribution. The variance of the error term can be given by:

\[
\text{Var}(\varepsilon) = \text{Var}(y) - \beta_1^2 \text{Var}(S) \tag{2.30}
\]

Since individual SNP usually contributes to a very small explained variance, the residual variance of \( y \) given \( S \) is very close to the total variance of \( y \). For each SNP \( S \), we have:

\[
E(S) = 2q(1 - q) + 2q^2 \tag{2.31}
\]

\[
\text{Var}(S) = 2q(1 - q) \tag{2.32}
\]

Based on equation 2.29, we could compute the expected trait value for a given genotype, i.e.,

\[
E(y|S = 0) = \beta_0 \tag{2.33}
\]

\[
E(y|S = 1) = \beta_0 + \beta_1 \tag{2.34}
\]

\[
E(y|S = 2) = \beta_0 + 2\beta_1 \tag{2.35}
\]

Where \( \beta_0 \) can be calculated by :

\[
\beta_0 = E(y) - \beta_1 E(S)
\]

Given the genotype, the quantitative trait is assumed to follow a normal distribution. Since the mean [2.33 to 2.35] and variance [2.30, or approximate by \( \text{var}(y) \)] of the normal distribution are both known, we can infer the probability that the trait value will exceed (or fall below) certain threshold(s).
Then we could infer the corresponding odds for a clinically category (e.g. high LDL-C) for a given genotype, i.e., \( \text{odds}_{S=0} = \frac{P(y|S=0)}{1-P(y|S=0)} \), \( \text{odds}_{S=1} = \frac{P(y|S=1)}{1-P(y|S=1)} \) and \( \text{odds}_{S=2} = \frac{P(y|S=2)}{1-P(y|S=2)} \). To estimate the coefficient \( \beta^b_1 \) when clinically defined categories of a quantitative trait are considered as the outcome, we could evaluate the above at \( S = 1, 2 \) and weigh them by their relative population frequency, i.e.,

\[
\beta^b_1 = \frac{2q(1-q) - \log \frac{\text{odds}_{S=2}}{\text{odds}_{S=0}}}{2q(1-q) + q^2 - \log \frac{\text{odds}_{S=1}}{\text{odds}_{S=0}}} + \frac{q^2}{2q(1-q) + q^2 - \log \frac{\text{odds}_{S=2}}{\text{odds}_{S=0}}}
\]

2.39

We may then use the delta method to calculate standard error of \( \beta^b_1 \).

Computing probability of comorbidity \( P_1 \) and \( P_2 \)

Our calculations require specifying the probability of comorbidity, \( E(P_1, P_2) \), for conversion of the linear coefficient back to the logistic scale in the final step of the algorithm. This measure is also required when the methodology is to be applied to three or more traits. While estimates could be obtained from related literature, the lifetime probability for comorbidity could be relatively hard to find.

If this is the case, we can calculate it from the OR (or relative risk, RR) of trait \( P_1 \) given the other trait \( P_2 \) (in which \( P_2 \) can be considered a risk factor). For example, one may obtain the OR of CAD given diabetes based on literature search or other means. Based on Bayes rule, we have:

\[
E(P_1, P_2) = \Pr(P_1 = 1 and P_2 = 1) = \Pr(P_1 = 1|P_2 = 1) \times \Pr(P_2 = 1)
\]

2.25

Here we shall develop an approach to compute \( \Pr(P_1 = 1|P_2 = 1) \) given OR or RR. Let \( f_{RF0} \) be the probability of having the disease (\( P_1 \)) given the absence of the risk factor (\( P_2 = 0 \)), \( f_{RF1} \) be the probability of having the disease (\( P_1 \)) given the presence of the risk factor (\( P_2 = 1 \)), \( P_{RF0} \) denote the probability of having no risk factor i.e. \( \Pr(P_2=0) \), and \( P_{RF1} \) denote the probability of having the risk factor i.e. \( \Pr(P_2=1) \).

From 2.25, we may also express \( E(P_1, P_2) \) as \( f_{RF1} \times P_{RF1} \).

Note that we have \( K = f_{RF0} P_{RF} + f_{RF0} RR \cdot P_{RF1} \) or

\[
f_{RF0} = K/(P_{RF0} + RR \cdot P_{RF1})
\]

2.26

When the RR of disease given the risk factor is available, the calculation is straightforward as by definition we have \( f_{RF1} = RR \cdot f_{RF0} \) and \( E(P_1, P_2) = f_{RF1} \times P_{RF1} \). The case is more complicated when only OR are available. Here we present an iterative procedure to estimate \( E(P_1, P_2) \). In the first step we may use OR to approximate RR,

\[
f_{RF0} \approx K/(P_{RF0} + OR \cdot P_{RF1})
\]

2.27

From Zhang et al\(^{20} \), OR may be estimated from RR by

\[
RR \approx OR/(1 - f_{RF0} + f_{RF0} \cdot OR)
\]

2.28

The newly estimated RR from 2.28 can be substituted back into 2.26 to obtain a new estimate of \( f_{RF0} \). The algorithm is iterated until RR becomes stable (change in RR between iterations <1e-10).
Finally we can compute the probability of comorbidity by \( E(P_1, P_2) = f_{RF1} \times P_{RF1} \).

**Simulation study**

**Application to binary traits**

To verify the feasibility and efficacy of our proposed framework, we simulated different sets of genotype-phenotype data, with 300 SNPs \( (i.e. N_{snp} = 300; \text{coded as } 0, 1, 2) \) and two binary traits. As the proposed framework is a SNP-based analysis, the number of simulated SNPs shall not affect the validity of our simulations. For each simulated SNP, the allele frequency was randomly generated from a uniform distribution with a value range of \([0.05, 0.95]\). The number of subjects with each disorder \( (i.e., ncases) \) was set to \([10000, 20000, 50000, 100000]\) with a disease prevalence \( (K) \) of 10%. Here, ncases indicated the sample size of cases in the whole simulated population cohort. Sample size of the whole population \( (ntotal) \) was estimated by \( ntotal = \frac{ncases}{K} \). Then, based on the generated allele frequencies, we could infer the genetic profiles for the whole simulated population cohort. The total SNP-based heritability \( (h^2) \) for each trait was set at 0.2 to 0.4, distributed among all SNPs. More specifically, we simulated standard normal variables \( z_i \sim N(0,1) \), and set mean effect size \( \mu = \frac{h^2}{\sqrt{N_{snp}}} \). The actual effect size for SNP \( i \) was set at \( \beta_i = \mu z_i \). The total liability \( y \) equals the sum of effects from each SNP plus a residual \( (e) \), i.e., \( y = \sum \beta_i x_i + e; \) the total variance of \( y \) was set to one. Following the liability threshold model, subjects with total liability exceeding a certain threshold \( [= \Phi^{-1}(K), \text{where } K \text{ is the disease prevalence}] \) are regarded as having the trait or disease. The non-shared genetic covariance between the two traits was set to 0.1.

From the simulated population cohort, we simulated two case-control studies with traits A and B as the outcome respectively. Suppose the number of cases for trait A and B in the population are respectively \( N_A \) and \( N_B \), and \( N = \max(N_A, N_B) \). For trait A, we picked \( N_A \) cases and \( 2N - N_A \) controls from the population. As for trait B, we picked \( N_B \) cases and \( 2N - N_B \) controls from the population. For comparison, we also simulated a ‘real’ comorbidity GWAS by picking all the comorbid cases \( (N_{comor}) \) in the simulated population cohort who are identified as having both trait A and B. Then \( (2N - N_{comor}) \) controls were included. Notably, case-control samples for two traits with different overlap rate \( (P) \) were simulated to demonstrate the feasibility of our proposed method. Here \( P \) was defined to be the ratio of overlapped samples (including overlapped cases \( N_{AB\_overlap} \) and controls \( N_{ctrl\_overlap} \)) and all picked samples for each case-control study, i.e., \( P = (N_{AB\_overlap} + N_{ctrl\_overlap}) / 2N \). To adjust the overlap rate, we need to increase or decrease the number of common cases and/or controls for both traits. Two different overlap rates were simulated for both comorbid and single disorder. Notably, most shared subjects were controls as they were far more abundant than cases.

We also considered another type of study design, namely a prospective study of a population. The simulation scheme is similar to the above, except that the controls consisted of the rest of the population who
were not cases. Again, we assessed the performance of our proposed method under different overlap rates. We first simulated completely overlapped cohorts for traits A and B. Then we simulated 50% overlapped cohorts, i.e., 50% cases and controls for trait B were from the cohort for trait A. Besides, we studied the performance of our proposed framework both for the case where all required parameters were given and for the case where disease prevalence was misspecified.

Transforming regression coefficients for quantitative traits to those based on clinically meaningful categories

We simulated datasets to verify the feasibility of our proposed approach to ‘transform’ regression coefficients of quantitative traits to coefficients based on clinically defined categories. The number of studied subjects for quantitative traits was set to [50000,100000]. For each simulated SNP, the allele frequency was randomly generated from a uniform distribution from [0.05,0.95]. SNP-heritability was set to 0.1 and 0.2. The aim was to compare the theoretical estimates of regression coefficients and SE (based on summary statistics alone) against those obtained from simulated raw genotype data.

Application to binary traits in cardiovascular medicine

We applied our approach to 4 cardiometabolic disorders/traits, namely coronary artery disease\(^{21}\) (CAD), type 2 diabetes mellitus\(^ {22}\) (T2DM), obesity\(^ {23}\) (BMI >=30) and stroke\(^ {24}\) (all types of stroke included), based on publicly available GWAS summary statistics. Details of these datasets are summarized in Table S1. Since the effect size of individual SNPs in different GWAS may not correspond to the same allele, we employed the ‘harmonise_data’ function in the package TwoSampleMR (https://mrcieu.github.io/TwoSampleMR) which integrates GWAS summary statistics from different sources, taking into account DNA strand issues\(^ {25-27}\). Analysis was performed for SNPs with MAF>=0.01. In total, we studied the genetic architectures of 18 disease ‘subtypes’ (6 are comorbidities and the remaining 12 are ‘single’ diseases without relevant comorbid conditions) and identified contributing genetic variants.

Genes, pathway and cell type enrichment analysis

To better understand the functional and biological mechanisms underlying the genetic component of these disease ‘subtypes’, we computed gene-based significance using MAGMA inserted in the web-based tool FUMA\(^ {28,29}\). We employed false discovery rate (FDR) for multiple testing correction and selected genes that with FDR<0.05 for further analysis. Also, we performed “tissue specificity” analysis by examining whether the susceptibility genes are differentially expressed in a particular tissue. Apart from these analyses, we also conducted pathway analysis using the program “ConsensusPathDB”, in order to unravel biological pathways that are unique to specific disease subtype and shared pathways among disease subtypes. Furthermore, we examined the cell types that are enriched for specific disease ‘subtypes’.

Finding heritability explained by common variants
To further understand the genetic architecture of the ‘subtypes’ of complex diseases, we calculated their SNP-based heritability by LD score regression (LDSR)\textsuperscript{30}. We also explored the genetic correlation between different disease subtypes with LDSR. Our aim is clarify whether disease “subtypes” are genetically different from each other.

**Mendelian Randomization (MR) analysis**

Mendelian Randomization (MR) is a methodology for inferring the causal relationship between risk factors and outcomes, using genetic variants as ‘instruments’ to represent the exposure. Here we performed two-sample MR in which the instrument-exposure and instrument-outcome associations were estimated in different samples. MR was conducted with ‘inverse-variance weighted’ (MR-IVW)\textsuperscript{31} and Egger regression (MR-Egger)\textsuperscript{25} approaches. One of the concerns of MR is horizontal pleiotropy, in which the genetic instruments have effects on the outcome other than through effects on the exposure. Note that MR-Egger is able to give valid estimates of causal effects in the presence of imbalanced horizontal pleiotropy. It could be assessed by whether the Egger intercept is significantly different from zero.

The IVW framework is widely used in MR. Here we used an IVW approach that is able to account for SNP correlations\textsuperscript{31}. A similar approach may be used for MR-Egger, which allows an intercept term in the weighted regression. Please refer to\textsuperscript{25} and the Supplementary Text for details. Inclusion of a larger panel of SNPs in partial LD may enable higher variance to be explained, thus improving the power of MR\textsuperscript{27}. Including “redundant” SNPs in addition to the causal variant(s) would not invalidate the results. However, including too many variants with high correlations may result in unstable causal estimates\textsuperscript{27}.

To ensure the robustness of our findings, we performed MR at multiple r\textsuperscript{2} thresholds (0.001, 0.01, 0.05, 0.1 and 0.15) and with SNP correlations taken into account. For simplicity, we mainly present the results at r\textsuperscript{2}=0.05. However, as we shall present later, our findings are consistent across various thresholds and full results are given in supplementary table.

Only SNPs which passed genome-wide significance (p<5E-8) were included as instruments. We employed the R packages “MendelianRandomization” (ver 0.4.1) and “TwoSampleMR” (ver 4.25) for analysis. If a SNP was not available in the outcome GWAS, we allow using a “proxy SNP” provided r\textsuperscript{2}>=0.8 with the original SNP. LD was extracted from the 1000 Genomes European samples.

**Multiple testing correction**

Multiple testing was corrected by the false discovery rate approach by Benjamini and Hochberg. The method controls the expected proportion false discoveries among the significant results.

**Results**

**Simulation results**
Simulation results for binary traits

Results of our simulation are presented in Tables 1 and 2. For more detailed simulation results, please refer to Table S2 and S3. The correlations between the estimated and actual coefficients were in general very high. Clearly, the correlation and RMSE improved with increased sample sizes and higher heritability explained by SNPs (i.e. with larger effect sizes of SNPs). Since current GWAS summary data are usually of very large sample sizes, often larger than 100,000, we believe the current method is sufficiently good to approximate the results from a GWAS of comorbid or other combination of diseases/traits. Also, the current method is valid under different rates of overlap between the input GWAS datasets.

In addition, it is obvious that the power increased with larger samples sizes (i.e., case sizes of corresponding traits) and heritability explained. The type I error is kept at or below 0.05 when results with \( p \leq 0.05 \) were considered significant. Interestingly, the power of the proposed analytic method is sometimes higher than the simulated actual GWAS with genotype data. This may be because only a small number of patients were affected with both diseases (\( N_{\text{comor}} \) is low); on the other hand, the number of subjects affected with either disease is relatively larger, so the two sets of case-control GWAS data (of traits A and B) may contain more information than a GWAS on the minority who are affected by both diseases.

Tables 3, 4 as well as S4 demonstrate the simulation results with misspecified population parameters for both comorbid and only single disorder. The proposed method is reasonably robust to misspecified population parameters.

Table 1 Simulation results for comorbid disorders compared with “real” GWAS

| Overlap rate | No. cases | \( H^2 \) A | \( H^2 \) B | Correlation | RMSE | Inferred | Real GWAS |
|---------------|-----------|--------------|--------------|-------------|-------|----------|----------|
|               |           | Beta | SE     | Beta | SE   | Type I error | Power | Type I error |
| Cases    | Controls |
| 0.08 0.15    | 10000     | 0.2  | 0.3   | 0.93095 | 0.90106 | 0.04914 | 0.02285 | 0.673 | 0.040 | 0.493 | 0.037 |
|            | 20000     | 0.2  | 0.3   | 0.96271 | 0.90086 | 0.03921 | 0.01645 | 0.757 | 0.037 | 0.630 | 0.040 |
|            | 50000     | 0.2  | 0.3   | 0.98470 | 0.90046 | 0.03131 | 0.01041 | 0.850 | 0.050 | 0.773 | 0.040 |
|            | 100000    | 0.2  | 0.3   | 0.99084 | 0.90200 | 0.02732 | 0.00739 | 0.873 | 0.047 | 0.810 | 0.023 |
|            | 10000     | 0.22 | 0.32  | 0.93668 | 0.88878 | 0.04894 | 0.02289 | 0.683 | ------ | 0.510 | ------ |
|            | 20000     | 0.22 | 0.32  | 0.96226 | 0.89106 | 0.04029 | 0.01642 | 0.773 | ------ | 0.637 | ------ |
|            | 50000     | 0.22 | 0.32  | 0.98538 | 0.89207 | 0.03249 | 0.01041 | 0.860 | ------ | 0.803 | ------ |
|            | 100000    | 0.22 | 0.32  | 0.99034 | 0.89560 | 0.02912 | 0.00739 | 0.883 | ------ | 0.817 | ------ |
| 0.04 0.4    | 10000     | 0.2  | 0.3   | 0.91762 | 0.89548 | 0.05188 | 0.02079 | 0.637 | 0.037 | 0.493 | 0.040 |
|            | 20000     | 0.2  | 0.3   | 0.96362 | 0.90304 | 0.04123 | 0.01511 | 0.770 | 0.030 | 0.627 | 0.033 |
|            | 50000     | 0.2  | 0.3   | 0.97820 | 0.90767 | 0.03188 | 0.00953 | 0.820 | 0.047 | 0.767 | 0.047 |
|            | 100000    | 0.2  | 0.3   | 0.98766 | 0.90933 | 0.02949 | 0.00682 | 0.870 | 0.047 | 0.827 | 0.027 |
Table 2 Simulation results for only single disorder compared with “real” GWAS

| Overlap rate | No. cases | $H^2_A$ | $H^2_B$ | Correlation | RMSE | Inferred | Real GWAS |
|--------------|-----------|---------|---------|-------------|------|----------|-----------|
|              |           | Beta    | SE      | Beta        | SE   | Power    | Type I    |
|              |           | SE      | Beta    | SE          | Power| Type I   | error     |
|              |           | Power   | Type I  | error       |      |          |           |
| 0.13 0.15    | 10000     | 0.2     | 0.3     | 0.95880     | 0.99865 | 0.02642 | 0.00027  | 0.617     | 0.043     | 0.583     | 0.030     |
|              | 20000     | 0.2     | 0.3     | 0.96924     | 0.99862 | 0.02325 | 0.00027  | 0.740     | 0.040     | 0.760     | 0.050     |
|              | 50000     | 0.2     | 0.3     | 0.98246     | 0.99881 | 0.01739 | 0.00018  | 0.823     | 0.040     | 0.807     | 0.040     |
|              | 100000    | 0.2     | 0.3     | 0.98633     | 0.99884 | 0.01533 | 0.00011  | 0.870     | 0.050     | 0.890     | 0.043     |
|              | 10000     | 0.22    | 0.32    | 0.96022     | 0.99843 | 0.02745 | 0.00030  | 0.657     | ------     | 0.600     | ------     |
|              | 20000     | 0.22    | 0.32    | 0.97404     | 0.99853 | 0.02274 | 0.00028  | 0.743     | ------     | 0.760     | ------     |
|              | 50000     | 0.22    | 0.32    | 0.98185     | 0.99874 | 0.01855 | 0.00019  | 0.830     | ------     | 0.840     | ------     |
|              | 100000    | 0.22    | 0.32    | 0.98527     | 0.99872 | 0.01653 | 0.00012  | 0.873     | ------     | 0.880     | ------     |
| 0.21 0.25    | 10000     | 0.2     | 0.3     | 0.95253     | 0.99815 | 0.02809 | 0.00047  | 0.617     | 0.037     | 0.580     | 0.033     |
|              | 20000     | 0.2     | 0.3     | 0.97124     | 0.99814 | 0.02271 | 0.00033  | 0.767     | 0.047     | 0.747     | 0.037     |
|              | 50000     | 0.2     | 0.3     | 0.98356     | 0.99827 | 0.01646 | 0.00022  | 0.823     | 0.043     | 0.807     | 0.033     |
|              | 100000    | 0.2     | 0.3     | 0.98407     | 0.99827 | 0.01645 | 0.00014  | 0.867     | 0.050     | 0.867     | 0.043     |
|              | 10000     | 0.22    | 0.32    | 0.95888     | 0.99766 | 0.02761 | 0.00051  | 0.657     | ------     | 0.610     | ------     |
|              | 20000     | 0.22    | 0.32    | 0.97158     | 0.99809 | 0.02323 | 0.00031  | 0.747     | ------     | 0.757     | ------     |
|              | 50000     | 0.22    | 0.32    | 0.98096     | 0.99818 | 0.01860 | 0.00022  | 0.833     | ------     | 0.820     | ------     |
|              | 100000    | 0.22    | 0.32    | 0.98517     | 0.99816 | 0.01657 | 0.00013  | 0.883     | ------     | 0.870     | ------     |

Note: here No. cases indicates the number of cases we defined for our simulation scenarios, $H^2$ indicates heritability, RMSE is abbreviated for root mean square error. Please refer to the main text for details on simulation methods. The ‘real’ GWAS was constructed by $N_{comor}$ cases and $(2N - N_{comor})$ controls.
Table 3 Simulation results for comorbid disorders with misspecified population parameters

| No. cases | 10000 | 10000 | 20000 | 20000 |
|-----------|-------|-------|-------|-------|
| Trait A | 0.1 | 0.1 | 0.12 | 0.12 |
| Trait B | 0.1 | 0.1 | 0.14 | 0.14 |
| Trait A | 0.1 | 0.1 | 0.16 | 0.16 |
| Trait B | 0.1 | 0.1 | 0.12 | 0.12 |
| Beta | 0.94209 | 0.94208 | 0.94177 | 0.96841 |
| SE | 0.93944 | 0.95796 | 0.97031 | 0.94076 |
| Correlations | 0.04325 | 0.04269 | 0.04895 | 0.03188 |
| RMSE | 0.02057 | 0.01647 | 0.01242 | 0.01435 |
| Power | 0.673 | 0.673 | 0.673 | 0.75 |
| Overlap rate | 100% | 100% | 100% | 100% |

Note: RMSE is abbreviated for root mean square error. Here the simulation results are based on whole population studies, thus the overlap rate indicates the overlap between two simulated populations for two traits. Original indicates the real disease prevalence, Misspecified indicates the ones we used for the inference of the statistics for comorbid disorder.

Table 4 Simulation results for only single disorder with misspecified population parameters

| No. cases | 10000 | 10000 | 20000 |
|-----------|-------|-------|-------|
| Trait A | 0.1 | 0.1 | 0.12 |
| Trait B | 0.1 | 0.1 | 0.14 |
| Trait A | 0.1 | 0.1 | 0.16 |
| Trait B | 0.1 | 0.1 | 0.16 |
| Beta | 0.99451 | 0.99439 | 0.99415 |
| SE | 0.99427 | 0.98758 | 0.97807 |
| Correlations | 0.02326 | 0.03874 | 0.05409 |
| RMSE | 0.00323 | 0.00668 | 0.01002 |
| Power | 0.667 | 0.667 | 0.667 |
| Overlap rate | 100% | 100% | 100% |

Note: RMSE is abbreviated for root mean square error. Here the simulation results are based on whole population studies, thus the overlap rate indicates the overlap between two simulated populations for two traits. Original indicates the real disease prevalence, Misspecified indicates the ones we used for the inference of the statistics for comorbid disorder.
Simulation results for clinically defined categories of quantitative trait

Table 5 demonstrates the simulation results for clinically defined categories of quantitative trait. Similar to binary traits, the correlation between estimated and actual coefficients are high. To be more specific, the correlation and RMSE improved with increased sample sizes and higher heritability explained by SNPs (i.e. with larger effect sizes of SNPs). Considering current summary data for continuous traits are usually of very large sizes, often larger than 100,000, our proposed method is sufficiently good to approximate the results of clinically defined categories from a GWAS of continuous traits.

Table 5 Simulation results for clinically defined categories of a continuous trait
Sample size | Case size | $H^2$ | Correlation | RMSE | Power | Type I error |
|---|---|---|---|---|---|---|
| | | | Beta | SE | Beta | SE | Inferred | Real GWAS | Inferred | Real GWAS |
| 50000 | 7635 | 0.1 | 0.96518 | 0.99905 | 0.01596 | 0.00701 | 0.657 | 0.497 | 0.023 | 0.033 |
| 50000 | 10336 | 0.1 | 0.97356 | 0.99936 | 0.01385 | 0.00612 | 0.657 | 0.507 | 0.023 | 0.027 |
| 50000 | 7719 | 0.2 | 0.98126 | 0.99825 | 0.01626 | 0.00707 | 0.783 | 0.627 | 0.023 | 0.033 |
| 50000 | 10455 | 0.2 | 0.98782 | 0.99894 | 0.01255 | 0.00545 | 0.783 | 0.653 | 0.023 | 0.023 |
| 100000 | 15789 | 0.1 | 0.98216 | 0.99909 | 0.01138 | 0.00503 | 0.727 | 0.607 | 0.043 | 0.030 |
| 100000 | 21103 | 0.1 | 0.98572 | 0.99966 | 0.00993 | 0.00417 | 0.727 | 0.650 | 0.047 | 0.063 |
| 100000 | 15773 | 0.2 | 0.99133 | 0.99825 | 0.01112 | 0.00503 | 0.817 | 0.727 | 0.043 | 0.030 |
| 100000 | 21345 | 0.2 | 0.99227 | 0.99894 | 0.01003 | 0.00387 | 0.817 | 0.773 | 0.043 | 0.063 |

Note: here Sample size indicates the sample size of our simulated dataset, Case size indicates number of cases based on our clinically defined categories, $H^2$ indicates heritability, RMSE is abbreviated for root mean square error.

Application to cardiovascular disorders/traits

The proposed framework was applied to 4 cardiovascular diseases/traits, the combination of which results in 18 disease ‘subtypes’ (6 are comorbidities and the remaining 12 are ‘single’ diseases without relevant comorbid conditions). We estimated the effect size (in terms of odds ratio comparing subjects with the disease ‘subtype’ versus those without) and the corresponding SE and p-values based on our presented analytic framework. Following the definition by the GWAS analytic platform FUMA, independent significant SNPs are defined as those that with $p<5e-8$ and are independent from each other at the default $r^2$ threshold ($r^2=0.6$). As for the definition of genomic loci, independent significant SNPs which are correlated with each other at $r^2 \geq 0.1$ are assigned to the same risk locus. Then independent significant SNPs which lie within 250 kb are merged into one genomic risk locus. As for the lifetime risk of these involved diseases, some of them were directly extracted from relevant literatures\(32-37\), while the remaining were inferred from odds ratios (or relative risks) from relevant studies (see supplementary Table S5).

Genes, cell type and pathway analysis

Here we report the analysis results for the 18 disease ‘subtypes’. In total, we identified 384 and 587 genomic risk loci respectively for 6 comorbidities and 12 disease ‘subtypes’ without a relevant comorbid condition (Table 6, Fig.1 and Table S6). Here we take Type 2 DM and obesity and the combination of these two traits as example. As expected, some susceptibility genes were shared among disease ‘subtypes’. For instance, $TCF7L2$ and $CDKAL1$\(38\) are the top susceptibility genes for all 3 disease subtypes involving T2DM and obesity\(39\). It is also worth noting that $TCF7L2$\(40-42\) and $CDKAL1$\(43,44\) are also among the top genes for all three disease subtypes involving CAD and T2DM, suggesting a more general role of these genes in the pathogenesis of various forms of cardiometabolic abnormalities. Some susceptibility genes were only identified in specific disease subtypes. For example, $FTO$ was found to be implicated only in disease subtypes...
that involved obesity, i.e., obesity with or without T2DM, but not T2DM without obesity. This finding is consistent with previous studies that FTO mainly contributes to diabetes through its effects on BMI. Interestingly, BDNF was among the top genes for obese DM. BDNF treatment has been shown to reduce weight gain and glucose level in animal models and was also associated with glucose metabolism in clinical studies. On the other hand, genes such as JAZF1, HMGA2, COBLL1, KCNJ11 and PPARG were ranked among the top for non-obese DM, indicating these genes may contribute to glucose dysregulation other than through effects on BMI/obesity. Notably, KCNJ11 and PPARG are also drug targets for sulphonylureas and thiazolidinediones (known anti-DM medications); further studies on the mechanisms and clinical efficacy of these classes of drugs in non-obese DM subjects may be warranted. For details about the concordant and discordant genes among disease subtypes, please refer to Table S7.

In addition, we also performed pathway analysis through the tool ConsensusPathDB. The enriched pathways were summarized in Table S8. Similar to gene analysis, some enriched pathways were shared among different disease subtypes while others were unique to particular disease subtype. Taking the 3 disease subtypes involving CAD and T2DM as example, statin pharmacodynamics, transcriptional regulation by RUNX3, and Angiopoietin receptor Tie2-mediated signaling were significantly enriched in all three disease subtypes, suggesting a broader role of these biological pathways across CVD. There were also pathways that were only significantly enriched in certain disease subtype. For example, amb2 Integrin signaling and chylomicron/plasma lipoprotein clearance were top-ranked for non-diabetic CAD. As another example, adipogenesis and MAPK cascade pathways were enriched in CAD comorbid with T2DM. Previous studies have implicated a role of MAPK cascade in the pathogenesis of cardiac diseases and diabetes.

By investigating the pathways enriched for each disease ‘subtype’, we hope to gain insight into biological mechanisms that are generally important across CV disorders, as well as more ‘specific’ mechanisms that may play a more salient role for certain disease combinations.

Next we also performed cell-type and tissue specificity analysis through FUMA. FUMA pre-computes a list of genes differentially expressed in different tissues (DEGs) from GTEx; input genes (significant genes from MAGMA analysis in GWAS) are then tested for enrichment for these DEGs. This approach is simple but differential expression does not always suggest causal role of the tissue. We consider this as a hypothesis-generating analysis. According to the tissue analysis results, coronary artery and aorta were the most significantly enriched tissues only for disease subtypes that involved CAD. Interestingly, for disease subtypes including obesity without CAD, obesity without stroke and non-obese T2DM, the most significantly enriched tissues includes brain tissues such as cerebellar hemisphere, cerebellum and frontal cortex (Fig. S2). While the results will require further experimental validation, the brain has been suggested to play a key role in the control of body fat content and glucose metabolism.
Recently methods have been developed for cell-type enrichment analysis based on GWAS, as single-cell sequencing data becomes more widely available. However, we note that single-cell data to date are more abundant for the brain than for other tissue types. This part is considered more exploratory as not all cell types are available for analysis in FUMA. We shall focus on the enrichment results for several comorbidities (most other disease combinations did not return significant results). To highlight a few interesting findings (Fig. S3), we found GABAergic neurons in the midbrain and prefrontal cortex to be the most enriched cell type for CAD with obesity. Interestingly, it has been reported that leptin exerts its anti-obesity effects mostly through GABAergic neurons in the brain. GABA neurotransmission is also thought to play a role in appetite regulation. On the other hand, GABA in the CNS may also regulate the sympathetic outflow to the coronary vasculature, causing a change in vascular resistance. For CAD with T2DM, we also found enrichment of endothelial cells in the pancreas.

Table 6  Genome-wide significant SNPs and risk loci for studied disease subtypes

| Disease subtypes      | No. of ind. Sig. SNPs | No. risk loci |
|-----------------------|-----------------------|---------------|
| CAD with T2DM         | 9695                  | 173           |
| CAD without T2DM      | 2582                  | 51            |
| T2DM without CAD      | 7727                  | 129           |
| CAD with Obesity      | 599                   | 22            |
| CAD without Obesity   | 446                   | 31            |
| Obesity without CAD   | 314                   | 17            |
| CAD with Stroke       | 1189                  | 34            |
| CAD without Stroke    | 1871                  | 33            |
| Stroke without CAD    | 271                   | 5             |
| T2DM with Obesity     | 1744                  | 69            |
| T2DM without Obesity  | 2911                  | 92            |
| Obesity without T2DM  | 316                   | 16            |
| T2DM with Stroke      | 2585                  | 72            |
| T2DM without Stroke   | 12260                 | 175           |
| Stroke without T2DM   | 247                   | 14            |
| Obesity with Stroke   | 359                   | 14            |
| Obesity without Stroke| 412                   | 18            |
| Stroke without Obesity| 111                   | 6             |

Note: here No. of ind. Sig. SNPs indicates number of independent significant SNPs

Heritability explained and genetic correlation among subtypes

In order to uncover how much variance could be explained by all common variants in the GWAS panel, we calculated the SNP-based heritability of our studied disease combinations by LD score regression. As
demonstrated in Table 6, almost all comorbid cardiometabolic traits are more heritable than only single traits (without a comorbid disorder).

We also assessed the genetic correlation between different disease ‘subtypes’ as defined by the presence or absence of comorbid conditions. The comparison results are summarized in Table 7. Interestingly, many pairs had weak or moderate genetic correlations, implying that they are possibly distinct biological subtypes of the disease. For example, comorbid CAD/T2DM only has a weak genetic correlation with non-diabetic CA (rg = 0.111), indicating that they may be genetically and biologically distinct ‘subtypes’. Similarly, only a moderate correlation was observed between obese CAD and non-obese CAD (rg = 0.232). Furthermore, we compared the extent of overlap of significant genetic variants among different pairs of disease subtypes. As expected, the weaker the genetic correlation, the lesser the overlap of significant SNPs between disease subtypes.

Genetic correlation and MR analysis

To further explore the genetic overlap between the studied disease subtypes and other cardiometabolic conditions (mainly stroke/CAD), we analyzed their genetic correlations using LD score regression (LDSR) (Table S9). This is also clinically relevant as we are often interested in whether a certain combination of traits is a significant risk factor for a certain disease. For example, do obese DM and non-obese DM confer the same risk to CAD? The findings will have implications for management and prevention of CAD. Here we performed LDSR and MR on several selected traits with higher clinical relevance. Table 8 shows the results from LDSR. For example, we observed that similar genetic correlation between obese and non-obese DM with CAD, suggesting the extent of genetic overlap with CAD are similar. On the other hand, the genetic correlation between obesity (without T2DM) per se and CAD is relatively weak (rg = 0.0797). As another example, while T2DM with obesity is moderately genetically correlated with stroke (rg = 0.2779), obesity without T2DM has no significant genetic correlation with stroke.

We then performed further MR analysis for selected pairs of traits to assess causal relationships between several disease subtypes and cardiovascular outcomes (Table 10 and S10). For simplicity, we primarily report the results at r² = 0.05, but most results are consistent across different r² thresholds. When focusing on CAD as the outcome, we found that obese DM is casually related to increased risk of CAD (MR-IVW; OR= 1.24, 95% CI: 1.16 to 1.33, p = 2.62E-11, Egger intercept p=0.355). Since we are studying binary exposures, the above OR roughly reflects the effect size with 2.72-fold increase in the exposure prevalence. Alternatively, the estimate may be multiple by 0.693 to reflect the OR resulted from doubling the prevalence of exposure, which is presented in our tables. Similar results were observed at other r² thresholds and with MR-Egger. Interestingly, we observed that obesity without DM does not have a significant causal link with CAD risks. On the other hand, non-obese DM showed no evidence of causal association under MR-Egger, but results were significant with MR-IVW. The Egger intercepts were significant (p<0.05 at all r2 thresholds), suggesting that
there is imbalanced horizontal pleiotropy, and that results from MR-Egger are more likely valid. The above finding suggests that some genetic variants may affect both non-obese DM and CAD risks via different pathways, leading to association between the two traits but the link may not be causal.

When considering stroke as the outcome, most disease subtypes studied were significantly and casually related to increased stroke risks. An exception is obesity alone without CAD or DM, which did not show a causal relationship with stroke. It is also worthwhile to note that the effect size also differs across different risk factors. For example, CAD comorbid with DM confers a higher risk (OR~ 1.12 per doubling of exposure prevalence; r² = 0.05) for stroke than DM without CAD (OR~ 1.03 per doubling of exposure prevalence) or CAD without DM (OR ~ 1.06 per doubling of exposure prevalence).

Table 7 Heritability of 18 disease combinations from LD score regression on the observed scale

| Comorbidities          | Heritability | Single Trait | Heritability | Single Trait | Heritability |
|------------------------|--------------|--------------|--------------|--------------|--------------|
| CAD with T2DM          | 0.0747 (0.0035) | CAD without T2DM | 0.0232 (0.0017) | T2DM without CAD | 0.0525 (0.0033) |
| CAD with Obesity       | 0.1562 (0.0071) | CAD without Obesity | 0.1014 (0.0069) | Obesity without CAD | 0.1168 (0.0059) |
| CAD with Stroke        | 0.0424 (0.0028) | CAD without Stroke | 0.0365 (0.0027) | Stroke without CAD | 0.0126 (0.0018) |
| T2DM with Obesity      | 0.0681 (0.0027) | T2DM without Obesity | 0.0421 (0.0032) | Obesity without T2DM | 0.0241 (0.0014) |
| T2DM with Stroke       | 0.0296 (0.0016) | T2DM without Stroke | 0.0568 (0.003) | Stroke without T2DM | 0.0077 (0.0009) |
| Obesity with Stroke    | 0.0511 (0.0027) | Obesity without Stroke | 0.0574 (0.0028) | Stroke without Obesity | 0.0221 (0.0022) |

Table 8 Genetic correlation of different disease subtypes

| Subtype 1               | Subtype 2               | rg    | P           | No. overlapped sig. SNPs |
|-------------------------|-------------------------|-------|-------------|--------------------------|
| T2DM with Obesity       | T2DM without Obesity   | 0.667 | **8.304E-170** | 335                      |
| T2DM with Obesity       | Obesity without T2DM   | 0.5568| **6.059E-108** | 290                      |
| CAD with T2DM           | CAD without T2DM       | 0.111 | **0.0044**   | 638                      |
| CAD with T2DM           | T2DM without CAD       | 0.6888| **6.12E-198** | 3333                     |
| CAD with Obesity        | CAD without Obesity    | 0.2324| **7.11E-12**  | 115                      |
| CAD with Obesity        | Obesity without CAD    | 0.5686| **2.13E-158** | 274                      |
| CAD with Stroke         | CAD without Stroke     | 0.9802| **2.38E-153** | 325                      |
| CAD with Stroke         | Stroke without CAD     | 0.3756| **2.03E-12**  | 117                      |
| T2DM with Stroke        | T2DM without Stroke    | 0.9348| **0.00E+00**  | 1845                     |
| T2DM with Stroke        | Stroke without T2DM    | 0.1343| **1.57E-02**  | 172                      |
| Obesity with Stroke     | Obesity without Stroke | 0.6711| **4.36E-306** | 282                      |
| Obesity with Stroke     | Stroke without Obesity | 0.4096| **7.6493E-21** | 9                        |

Note: here rg indicates the genetic correlation between two traits. No. overlapped sig. SNPs indicates the number of SNPs with P < 5E-8 in both disease subtypes.
Table 9  Genetic correlations between studies disease subtypes and other cardiometabolic conditions

| Disease subtypes          | Conditions | rg    | gcov  | P          |
|---------------------------|------------|-------|-------|------------|
| T2DM with Obesity         | CAD        | 0.357 | 0.0254| 2.571E-46  |
| T2DM without Obesity      | CAD        | 0.347 | 0.016 | 3.2467E-30 |
| Obesity without T2DM      | CAD        | 0.0797| 0.007 | 0.0241     |
| CAD with T2DM             | Stroke     | 0.4737| 0.0537| 9.1061E-39 |
| CAD without T2DM          | Stroke     | 0.2941| 0.0018| 6.4598E-09 |
| T2DM without CAD          | Stroke     | 0.1368| 0.0328| 0.0005     |
| T2DM with Obesity         | Stroke     | 0.2779| 0.0303| 1.0613E-15 |
| T2DM without Obesity      | Stroke     | 0.3103| 0.028 | 1.6109E-11 |
| CAD with Obesity          | Stroke     | 0.3595| 0.0201| 1.509E-18  |
| CAD without Obesity       | Stroke     | 0.3585| 0.0093| 1.45E-11   |
| Obese without CAD         | Stroke     | -0.0126| 0.0061| 0.7892     |

Note: here rg indicates the genetic correlation between two traits, gcov indicates the intercept for genetic covariance calculation.

Table 10  MR analysis results of selected pairs of traits.

| Exposure                          | Outcome | Estimate  | CILower | CIUpper | Pvalue | Method |
|-----------------------------------|---------|-----------|---------|---------|--------|--------|
| T2DM without Obesity             | CAD     | 1.43E 03  | -6.61E-02| 6.32E-02| 9.65E-01| Egger  |
| CAD with T2DM                     | Stroke  | 1.64E 01  | 1.29E-01 | 1.98E-01| 3.36E-20| IVW    |
| T2DM with Obesity                 | CAD     | 2.21E 01  | 1.56E-01 | 2.87E-01| 2.62E-11| IVW    |
| T2DM without Obesity              | Stroke  | 7.77E 02  | 4.83E-02 | 1.07E-01| 2.32E-07| IVW    |
| T2DM without CAD                  | Stroke  | 4.63E 02  | 2.29E-02 | 6.97E-02| 1.06E-04| IVW    |
| CAD without Obesity               | Stroke  | 1.20E 01  | 5.03E-02 | 1.90E-01| 7.59E-04| IVW    |
| CAD with Obesity                  | Stroke  | 1.02E 01  | 3.95E-02 | 1.65E-01| 1.43E-03| IVW    |
| T2DM with Obesity                 | Stroke  | 9.99E 02  | 3.63E-02 | 1.63E-01| 2.07E-03| IVW    |
| CAD without T2DM                  | Stroke  | 8.16E 02  | 2.15E-02 | 1.42E-01| 7.82E-03| IVW    |
| Obesity without T2DM              | CAD     | 7.46E 02  | -2.96E-02| 1.79E-01| 1.60E-01| IVW    |
| Obesity without T2DM              | Stroke  | 3.39E 02  | -1.85E-02| 8.64E-02| 2.05E-01| IVW    |
| Obesity without CAD               | Stroke  | 3.99E 03  | -6.27E-02| 7.07E-02| 9.07E-01| IVW    |

Note: here CILower indicates the lower bound for the confidence interval, CIUpper indicates the upper bound for the confidence interval.

If the Egger intercept p-value was <0.05, the Egger regression approach was used; otherwise we employed the MR-IVW approach which has better statistical power.

**Extension to more than two traits**

As discussed above, our analytic framework may also be applied to the combination of three or more traits. To illustrate the methodology, we applied it to three cardiometabolic disorders, namely CAD, T2DM and obesity. Specifically, we explored the genetic architecture of obese T2DM comorbid with CAD, and non-obese T2DM with CAD. In brief, we applied the analytic method sequentially by first deriving the GWAS results of DM with and without obesity, then adding CAD as input in the next step.
Accordingly, we identified 76 and 91 genomic risk loci respectively for obese T2DM with CAD and non-obese T2DM with CAD that exceed genome-wide significance (Table S6). Details about gene and pathway analysis results were summarized in Table S7 and S8. Similar to our results above, many susceptibility genes and enriched pathways were implicated in relevant pathophysiological process for the involved cardiometabolic diseases, and some genes/pathways are shared between the subtypes while some are top-ranked for specific subtypes. For a brief highlight of the results, for example, TCF7L2 and CDKN2B were among the top susceptible genes for both disease subtypes, while BDNF and HMGA2 were only found to be susceptible in obese T2DM+CAD and non-obese T2DM+CAD respectively. As for pathways, plasma lipoprotein assembly, remodeling and clearance was one of the top enriched pathways unique to obese T2DM with CAD while anti-diabetic drug potassium channel inhibitors pathway was only found to be significantly enriched in the other disease subtype, i.e., non-obese T2DM with CAD.

Application to clinically defined categories

Furthermore, we applied our proposed framework to low-density lipoprotein cholesterol (LDL) based on publicly available summary statistics with a sample size of 188,577\(^61\). Typically, a LDL cholesterol level reading of 190 mg/dL or higher is considered as very high in clinical practice. Following this standard, we transformed the summary statistics of quantitative trait into that of binary trait. Then, we uncovered the genetic architectures of disease combinations involved CAD and high LDL utilizing our proposed framework. Totally, we identified 80, 40 and 65 genomic risk loci that exceed genome-wide significance respectively for CAD with high LDL, CAD without high LDL and high LDL without CAD. For details about these genomic risk loci, please refer to Table S6. Genes and pathways analysis results were demonstrated in Table S7 and S8. Analysis results suggest that identified susceptible genes and enriched pathways were strongly linked to the pathophysiology of involved disorders\(^62-64\). As for tissue specificity analysis, we found that liver was the most significantly enriched tissue for CAD with high LDL.

Discussion

Here we have presented a statistical framework to uncover susceptibility variants for combination of diseases/traits, based on summary statistics alone. The method is useful for revealing the genetic basis of comorbid disorders, or a disorder without comorbidity. More broadly speaking, the cases can be considered as those affected by as specific ‘subtype’ of the disease (as characterized by the presence or absence of comorbid traits). We also extended the methodology to deal with continuous traits with clinically meaningful categories (e.g. lipid levels), and to more than 2 traits.

There are several strengths and potential applications of the proposed framework. Firstly, as the method only requires GWAS summary statistics, our framework can be potentially applied to a large variety of complex diseases. This approach is likely more cost-effective than recruiting subjects with comorbid disorders.
As GWAS summary statistics with large sample sizes have dramatically increased these years, we believe the proposed framework represents a new paradigm and will open up countless opportunities to study the genetic basis and architecture of disease combinations. Such efforts will help shed light on heterogeneity and pathophysiology of different complex disorders, and may contribute to the identification of new drug targets and more personalized therapies. As for other clinical implications, different ‘subtypes’ of a disease may be related to different complications. For example, we found that obese DM is causally related to increased risks of CAD, but obesity without DM is not significantly linked to CAD. Similarly, LDSR also showed a weak genetic correlation between obesity with no DM and CAD. These analyses will help to refine causal risk factors for diseases and the formulation of prevention strategies. Note that other secondary analysis of GWAS summary data, such as transcriptome-wide association studies (TWAS), Summary-data-based Mendelian Randomization (SMR; based on eQTLs, methylation QTLs etc.), other SNP-based (partitioned) heritability estimation, pathway analysis approaches etc. may also be applied although we only illustrate the application of a few methods.

There are a few limitations to the current study. Similar to other methodologies that employ summary statistics from more than one sample, such as two-sample MR, there is an implicit assumption that both sets of summary statistics (assuming the study of 2 traits) are based on the same population. Large heterogeneity between the samples (e.g. different ethnic groups, large differences in inclusion/exclusion criteria etc.) may lead to inaccurate results. Also, most available summary statistics to date, including those we included in this study, are based on European samples. The results may not be transferable to other populations and it remains an open question how to accommodate summary data from different populations. While we believe the proposed framework is flexible and cost-effective, it could not completely replace the need to recruit subjects with comorbidities (or diseases without comorbidity). As discussed above, heterogeneity between study samples is inevitable, and recruitment of a more homogeneous sample with detailed phenotyping is still very valuable in uncovering the genetic basis of combination of diseases. While we highlights several genes and pathways underlying cardiometabolic traits, further experimental studies are required to validate the findings.

Taken together, we believe the proposed approach is a useful extension to conventional single-trait analysis. Identification of genetic variants for comorbid disorders or disease ‘subtypes’ may ultimately lead to more targeted prevention and treatment, and identification of novel drug targets.

**Supplementary Materials are available at**

https://drive.google.com/open?id=1Q_FGQIslb5MY6pOx6_5IXy3Gi8CgQL2s

**Acknowledgements**
This work was supported partially by the Lo Kwee Seong Biomedical Research Fund, a Direct Grant from The Chinese University of Hong Kong, RGC Collaborative Research Fund C4054-17WF and an NSFC grant. We are grateful to Prof. Stephen Tsui for computing support.

**Author contributions** Conceived and designed the study: HCS. Study supervision: HCS. Data analysis: LYY (lead), with input from CKLC and YPL. Methodology: HCS, LYY. Data interpretation: HCS, LYY. Drafted the manuscript: LYY, HCS.

**Conflicts of interest**

The authors declare no conflict of interest.

**Figure legends**

Figure 1 Manhattan plot of GWAS results of comorbid disorders and single disorder without comorbidity

**References**

1. Deloukas P, Kanoni S, Willenborg C, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*. 2013;45(1):25.

2. Gratten J, Wray NR, Keller MC, Visscher PM. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nat Neurosci*. 2014;17(6):782.

3. Zhao K, So H. Drug repositioning for schizophrenia and depression/anxiety disorders: A machine learning approach leveraging expression data. *IEEE journal of biomedical and health informatics*. 2018;23(3):1304-1315.

4. So H, Chau CK, Chiu W, et al. Analysis of genome-wide association data highlights candidates for drug repositioning in psychiatry. *Nat Neurosci*. 2017;20(10):1342.

5. Yin L, Chau CK, Sham P, So H. Integrating clinical data and imputed transcriptome from GWAS to uncover complex disease subtypes: Applications in psychiatry and cardiology. *The American Journal of Human Genetics*. 2019;105(6):1193-1212.
6. Yin L, Cheung EF, Chen RY, Wong EH, Sham P, So H. Leveraging genome-wide association and clinical data in revealing schizophrenia subgroups. *J Psychiatr Res*. 2018;106:106-117.

7. Kooperberg C, LeBlanc M, Obenchain V. Risk prediction using genome-wide association studies. *Genet Epidemiol*. 2010;34(7):643-652.

8. Agerbo E, Sullivan PF, Vilhjálmsdóttir BJ, et al. Polygenic risk score, parental socioeconomic status, family history of psychiatric disorders, and the risk for schizophrenia: A danish population-based study and meta-analysis. *JAMA psychiatry*. 2015;72(7):635-641.

9. Buckley PF, Miller BJ, Lehrer DS, Castle DJ. Psychiatric comorbidities and schizophrenia. *Schizophr Bull*. 2008;35(2):383-402.

10. Bramante CT, Lee CJ, Gudzune KA. Treatment of obesity in patients with diabetes. *Diabetes Spectrum*. 2017;30(4):237-243.

11. Hirschfeld RM. The comorbidity of major depression and anxiety disorders: Recognition and management in primary care. *Primary care companion to the Journal of clinical psychiatry*. 2001;3(6):244.

12. Benjamin EJ, Virani SS, Callaway CW, et al. Heart disease and stroke statistics-2018 update: A report from the american heart association. *Circulation*. 2018;137(12):e67.

13. Nieuwboer HA, Pool R, Dolan CV, Boomsma DJ, Nivard MG. GWIS: Genome-wide inferred statistics for functions of multiple phenotypes. *The American Journal of Human Genetics*. 2016;99(4):917-927.

14. Bulik-Sullivan BK, Loh P, Finucane HK, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015;47(3):291.

15. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS genetics*. 2014;10(5):e1004383.
16. Papan Thaipisuttikul PI, Waleeprakhon P, Wisajun P, Jullagate S. Psychiatric comorbidities in patients with major depressive disorder. *Neuropsychiatric disease and treatment*. 2014;10:2097.

17. Lloyd-Jones LR, Robinson MR, Yang J, Visscher PM. Transformation of summary statistics from linear mixed model association on all-or-none traits to odds ratio. *Genetics*. 2018;208(4):1397-1408.

18. Prentice RL, Pyke R. Logistic disease incidence models and case-control studies. *Biometrika*. 1979;66(3):403-411.

19. Oehlert GW. A note on the delta method. *The American Statistician*. 1992;46(1):27-29.

20. Zhang J, Kai FY. What's the relative risk?: A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA*. 1998;280(19):1690-1691.

21. Nelson CP, Goel A, Butterworth AS, et al. Association analyses based on false discovery rate implicate new loci for coronary artery disease. *Nat Genet*. 2017;49(9):1385.

22. Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat Genet*. 2018;50(11):1505.

23. Berndt SI, Gustafsson S, Mägi R, et al. Genome-wide meta-analysis identifies 11 new loci for anthropometric traits and provides insights into genetic architecture. *Nat Genet*. 2013;45(5):501.

24. Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50(4):524.

25. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: Effect estimation and bias detection through egger regression. *Int J Epidemiol*. 2015;44(2):512-525.

26. Davey Smith G, Hemani G. Mendelian randomization: Genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23(R1):R89-R98.
27. Pierce BL, Burgess S. Efficient design for mendelian randomization studies: Subsample and 2-sample instrumental variable estimators. *Am J Epidemiol.* 2013;178(7):1177-1184.

28. Watanabe K, Taskesen E, Van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nature communications.* 2017;8(1):1826.

29. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: Generalized gene-set analysis of GWAS data. *PLoS computational biology.* 2015;11(4):e1004219.

30. Bulik-Sullivan BK, Loh P, Finucane HK, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet.* 2015;47(3):291.

31. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* 2016;40(4):304-314.

32. Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A. Epidemiology of coronary heart disease and acute coronary syndrome. *Annals of translational medicine.* 2016;4(13).

33. Lifetime risk. [https://www.snpedia.com/index.php/Lifetime_Risk](https://www.snpedia.com/index.php/Lifetime_Risk). Updated 2019.

34. Schableger K, Inreiter L. Incidence of stroke in the diabetic and non-diabetic population in upper austria (2008-2012) and. *Austrian Journal of Statistics.* 2015;44(3):69-83.

35. Calvet D, Touzé E, Varenne O, Sablayrolles J, Weber S, Mas J. Prevalence of asymptomatic coronary artery disease in ischemic stroke patients. *The PRECORIS Study Circulation.* 2010;121:1623.

36. Kivimäki M, Kuosma E, Ferrie JE, et al. Overweight, obesity, and risk of cardiometabolic multimorbidity: Pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and europe. *The Lancet Public Health.* 2017;2(6):e277-e285.

37. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: A systematic review and meta-analysis. *BMC Public Health.* 2009;9(1):88.
38. Palmer CJ, Bruckner RJ, Paulo JA, et al. Cdkal1, a type 2 diabetes susceptibility gene, regulates mitochondrial function in adipose tissue. *Molecular metabolism*. 2017;6(10):1212-1225.

39. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007;445(7130):881.

40. Gloyn AL, Braun M, Rorsman P. Type 2 diabetes susceptibility gene TCF7L2 and its role in β-cell function. *Diabetes*. 2009;58(4):800-802.

41. Sousa AGP, Marquezine GF, Lemos PA, et al. TCF7L2 polymorphism rs7903146 is associated with coronary artery disease severity and mortality. *PLoS One*. 2009;4(11):e7697.

42. Srivastava R, Zhang J, Go G, Narayanan A, Nottoli TP, Mani A. Impaired LRP6-TCF7L2 activity enhances smooth muscle cell plasticity and causes coronary artery disease. *Cell reports*. 2015;13(4):746-759.

43. Dehwah MA, Wang M, Huang QY. CDKAL1 and type 2 diabetes: A global meta-analysis. *Genet Mol Res*. 2010;9(2):1109-1120.

44. Saade S, Cazier J, Ghassibe-Sabbagh M, et al. Large scale association analysis identifies three susceptibility loci for coronary artery disease. *PloS one*. 2011;6(12):e29427.

45. Freathy RM, Timpson NJ, Lawlor DA, et al. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes*. 2008;57(5):1419-1426.

46. Kamura Y, Iwata M, Maeda S, et al. FTO gene polymorphism is associated with type 2 diabetes through its effect on increasing the maximum BMI in japanese men. *PloS one*. 2016;11(11):e0165523.

47. Yang Y, Liu B, Xia W, et al. FTO genotype and type 2 diabetes mellitus: Spatial analysis and meta-analysis of 62 case-control studies from different regions. *Genes*. 2017;8(2):70.
48. Eyileten C, Kaplon-Cieslicka A, Mirowska-Guzel D, Malek L, Postula M. Antidiabetic effect of brain-derived neurotrophic factor and its association with inflammation in type 2 diabetes mellitus. *Journal of diabetes research*. 2017;2017.

49. Wang Y, Gao H, Shi C, et al. Leukocyte integrin mac-1 regulates thrombosis via interaction with platelet GPIIbα. *Nature communications*. 2017;8:15559.

50. Castro Cabezas M, Botham KM, Mamo JC, Proctor SD. Novel aspects of nonfasting lipemia in relation to vascular biology. *International Journal of Vascular Medicine*. 2012;2012.

51. Wang Y. Mitogen-activated protein kinases in heart development and diseases. *Circulation*. 2007;116(12):1413-1423.

52. Muslin AJ. MAPK signalling in cardiovascular health and disease: Molecular mechanisms and therapeutic targets. *Clin Sci*. 2008;115(7):203-218.

53. Haneda M, Araki S, Togawa M, Sugimoto T, Motohide I, Kikkawa R. Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes*. 1997;46(5):847-853.

54. Schwartz MW, Porte D. Diabetes, obesity, and the brain. *Science*. 2005;307(5708):375-379.

55. Raji CA, Ho AJ, Parikshak NN, et al. Brain structure and obesity. *Hum Brain Mapp*. 2010;31(3):353-364.

56. Watanabe K, Mirkov MU, de Leeuw CA, van den Heuvel, Martijn P, Posthuma D. Genetic mapping of cell type specificity for complex traits. *Nature communications*. 2019;10(1):1-13.

57. Vong L, Ye C, Yang Z, Choi B, Chua Jr S, Lowell BB. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron*. 2011;71(1):142-154.

58. Delgado TC. Glutamate and GABA in appetite regulation. *Frontiers in endocrinology*. 2013;4:103.
59. Segal SA, Jacob T, Gillis RA. Blockade of central nervous system GABAergic tone causes sympathetic-mediated increases in coronary vascular resistance in cats. Circ Res. 1984;55(3):404-415.

60. Burgess S, Labrecque JA. Mendelian randomization with a binary exposure variable: Interpretation and presentation of causal estimates. Eur J Epidemiol. 2018;33(10):947-952.

61. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet. 2013;45(11):1274.

62. Talmud PJ, Drenos F, Shah S, et al. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. The American journal of human genetics. 2009;85(5):628-642.

63. Rossignoli A, Shang M, Gladh H, et al. Poliovirus Receptor–Related 2: A cholesterol-responsive gene affecting atherosclerosis development by modulating leukocyte migration. Arterioscler Thromb Vasc Biol. 2017;37(3):534-542.

64. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: Risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med. 2004;255(2):188-205.
Figure 1

- CAD with T2DM
- CAD without T2DM
- T2DM without CAD

- CAD with Obesity
- CAD without Obesity
- Obesity without CAD

- CAD with Stroke
- CAD without Stroke
- Stroke without CAD

- T2DM with Obesity
- T2DM without Obesity
- Obesity without T2DM

- T2DM with Stroke
- T2DM without Stroke
- Stroke without T2DM

- Obesity with Stroke
- Obesity without Stroke
- Stroke without Obesity
Table S1 Details about the datasets for GWAS summary statistics inference after quality control

| Disease combination    | No. SNPs  | Sample size of the 1st disease | Sample size of the 2nd disease |
|------------------------|-----------|---------------------------------|---------------------------------|
|                        |           | Cases                      | controls                      | Cases                      | controls                      |
| CAD with T2DM         | 7,720,471 | 10,801                      | 137,914                       | 74,124                     | 824,006                       |
| CAD with Obesity      | 2,274,450 | 10,801                      | 137,914                       | 32,858                     | 65,839                        |
| CAD with Stroke       | 7,612,115 | 10,801                      | 137,914                       | 67,162                     | 454,450                       |
| T2DM with Obesity     | 2,277,486 | 74,124                      | 824,006                       | 32,858                     | 65,839                        |
| T2DM with Stroke      | 7,431,694 | 74,124                      | 824,006                       | 67,162                     | 454,450                       |
| Obesity with Stroke   | 2,274,378 | 32,858                      | 65,839                        | 67,162                     | 454,450                       |
| CAD without T2DM      | 7,720,471 | 10,801                      | 137,914                       | 74,124                     | 824,006                       |
| CAD without Obesity   | 2,274,450 | 10,801                      | 137,914                       | 32,858                     | 65,839                        |
| CAD without Stroke    | 7,612,115 | 10,801                      | 137,914                       | 67,162                     | 454,450                       |
| T2DM without Obesity  | 2,277,486 | 74,124                      | 824,006                       | 32,858                     | 65,839                        |
| T2DM without Stroke   | 7,431,694 | 74,124                      | 824,006                       | 67,162                     | 454,450                       |
| T2DM without CAD      | 7,720,471 | 74,124                      | 824,006                       | 10,801                     | 137,914                       |
| Obesity without CAD   | 2,274,450 | 32,858                      | 65,839                        | 10,801                     | 137,914                       |
| Obesity without T2DM  | 2,277,486 | 32,858                      | 65,839                        | 74,124                     | 824,006                       |
| Obesity without Stroke| 2,274,378 | 32,858                      | 65,839                        | 67,162                     | 454,450                       |
| Stroke without CAD    | 7,612,115 | 67,162                      | 454,450                       | 10,801                     | 137,914                       |
| Stroke without T2DM   | 7,431,694 | 67,162                      | 454,450                       | 74,124                     | 824,006                       |
| Stroke without Obesity| 2,274,378 | 67,162                      | 454,450                       | 32,858                     | 65,839                        |

Table S2 Simulation results for comorbid disorders

Table S3 Simulation results for single disorder without comorbidity
Table S4 Simulation results for comorbid disorders and single disorders with misspecified population parameters

Table S5 Odds ratios of involved diseases extracted from relevant studies

| Disorders      | OR  |
|----------------|-----|
| CAD | Obesity | 1.5 |
| CAD | Stroke   | 3.7 |
| T2DM | Obesity | 9.4 |
| Stroke | Obesity | 1.5 |

Table S6 Identified susceptible genetic loci for studied disorders

Table S7 Shared and unique genes among different disease “subtypes” based on MAGMA

Table S8 Shared and unique pathways among different disease “subtypes” based on ConsensumPathDB

Table S9 Genetic correlations between studied diseases and other diseases

Table S10 MR analysis results for selected disorders

Figure S1 Figure showing that the absolute values of coefficient for binary traits derived from a linear regression is smaller than the coefficient (\( \alpha \)) under a logistic model

Figure S2 Results of tissue enrichment analysis

Figure S3 Results of cell-type enrichment for comorbid disorders