Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies

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Abstract

In genome-wide association studies (GWAS) for thousands of phenotypes in large biobanks, most binary traits have substantially fewer cases than controls. Both of the widely used approaches, linear mixed model and the recently proposed logistic mixed model, perform poorly -- producing large type I error rates -- in the analysis of phenotypes with unbalanced case-control ratios. Here we propose a scalable and accurate generalized mixed model association test that uses the saddlepoint approximation (SPA) to calibrate the distribution of score test statistics. This method, SAIGE, provides accurate p-values even when case-control ratios are extremely unbalanced. It utilizes state-of-art optimization strategies to reduce computational time and memory cost of generalized mixed model. The computation cost linearly depends on sample size, and hence can be applicable to GWAS for thousands of phenotypes by large biobanks. Through the analysis of UK-Biobank data of 408,961 white British European-ancestry samples, we show that SAIGE can efficiently analyze large sample data, controlling for unbalanced case-control ratios and sample relatedness.

Introduction

Decreases in genotyping cost allow for large biobanks to genotype all participants, enabling genome-wide scale phenome-wide association studies (PheWAS) in hundreds of thousands of samples. In a typical genome-wide PheWAS**,** GWAS for tens of million variants are performed for thousands of phenotypes constructed from Electronic Health Records (EHR) and/or survey questionnaires from participants in large cohorts[1–18]. For binary traits based on disease/condition status, cases are typically defined as individuals with specific International Classification of Disease (ICD) codes within the EHR. Controls are usually all participants without the same or other related conditions[1,3]. Due to the low prevalence of many conditions/diseases, case-control ratios are often unbalanced (case:control=1:10) or extremely unbalanced (case:control=1:100). The scale of data and the unbalanced nature of binary traits pose substantial challenges for genome-wide PheWAS in biobanks.

Population structure and relatedness are major confounders in genetic association studies and also need to be controlled in PheWAS. Linear mixed models (LMM) are widely used to account for these issues in GWAS for both binary and quantitative traits[19–25]. However, since LMM is not designed to analyze binary traits, it can have inflated type I error rates, especially in the presence of unbalanced case-control ratios. Recently, Chen, H. *et al*. have proposed to use logistic mixed models and developed a score test called the generalized mixed model association test (GMMAT)[26]. GMMAT assumes that score test statistics asymptotically follow a Gaussian distribution to estimate asymptotic p-values. Although GMMAT test statistics are more robust than the LMM based approaches, it can also suffer type I error rate inflation when case-control ratios are unbalanced, because unbalanced case-control ratios invalidate asymptotic assumptions of logistic regression. In addition, since GMMAT requires $O(MN^2)$ computation and $O(N^2)$ memory space, where M is the number of genetic variants to be tested and N is the number of individuals, it cannot handle data with hundreds of thousands of samples.

Here, we propose a novel method to allow for analysis of very large samples, for binary traits with unbalanced case-control ratios, which also infers and accounts for sample relatedness. Our method, Scalable and Accurate Implementation of GEneralized mixed model (SAIGE), uses the saddlepoint approximation (SPA)[27–29] to calibrate unbalanced case-control ratios in score tests based on logistic mixed models. Since SPA uses all the cumulants, and hence all the moments, it is more accurate than using the Gaussian distribution, which uses only the first two moments. Similar to BOLT-LMM[25], the large sample size method for linear mixed-models, our method utilizes state-of-art optimization strategies, such as the preconditioned conjugate gradient (PCG) approach[30,31] for solving linear systems for large cohorts without requiring a pre-computed genetic relationship matrix (GRM). The overall computation cost of this proposed method is $O(MN)$, which is substantially lower than the computation cost of GMMAT[26] and many popular LMM methods, such as GEMMA[24]. In addition, we reduce the memory use by compactly storing raw genotypes instead of calculating and storing the GRM.

We have demonstrated that SAIGE controls for the inflated type I error rates for binary traits with unbalanced case-control ratios in related samples through simulation and the UK Biobank data of 408,961 white British samples[32,33]. By evaluating its computation performance, we demonstrate the feasibility of SAIGE for large-scale PheWAS.

RESULTS

Overview of Methods

The SAIGE method contains two main steps: 1. Fitting the null logistic mixed model to estimate variance component and other model parameters. 2. Testing for association between each genetic variant and phenotypes by applying SPA to the score test statistics. Step 1 iteratively estimates the model parameters using the computational efficient average information restricted maximum likelihood (AI-REML) algorithm[34], which is also used in GMMAT[26]. Several optimization strategies have been applied in step 1 to make fitting the null logistic mixed model practical for large data sets, such as the UK Biobank [32,33]. First, the spectral decomposition has been replaced by the PCG to solve linear systems without inversing the $N \times N$ GRM[31] (as in BOLT-LMM[25]). The PCG method iteratively finds solutions of the linear system in a computation and memory efficient way. Thus, instead of requiring a pre-computed GRM, which costs a significant amount of time to calculate when sample sizes are large, SAIGE uses the raw genotypes as input. The computation time is about O(M_1N) times the number of iterations for the conjugate gradient to converge, where M_1 is a number of variants to be used for constructing GRM. Second, to further reduce the memory usage during the model fitting, the raw genotypes are stored in a binary vector and elements of GRM are calculated when needed rather than being stored, so the memory usage is $M_1N/4$ bytes (as in BOLT-LMM[25] and GenABEL[35]). For example, for the UK Biobank data with M_1 = 93,511 and N = 408,961 (white British participants), the memory usage drops from 669 Gigabytes(Gb) for storing the GRM with float numbers to 9.56 Gb for the raw genotypes in a binary vector.

After fitting the null logistic mixed model, the estimate of the random effects for each individual is obtained. The ratio of the variances of the score statistics with and without incorporating the variance components for the random effects is calculated using a subset of randomly selected genetic variants, similar to BOLT-LMM[25] and GRAMMAR-Gamma[36]. This ratio has been previously suggested to be constant for score tests based on LMMs[36]. We have shown that the ratio is also approximately constant for all genetic variants with MAC > 20 in the scenario of the logistic mixed models through analytic derivation and simulations (**Supplementary Methods and Supplementary Figure 1**).

In step 2, for each variant, the variance ratio is used to calibrate the score statistic variance that does not incorporate variance components for random effects. Since GRM is no longer needed for this step, the computation time to obtain the score statistic for each variant is O(N). SAIGE next approximates the score test statistics using the SPA to obtain more accurate p-values than the normal distribution. A faster version of the SPA test, similar to the fastSPA method in the SPAtest R package that we recently developed[29] , is used to further improve the computation time, which exploits the sparsity in low frequency or rare variants to reduce the computation cost.

Computation and Memory Cost

The key features of SAIGE compared to other existing methods are presented in **Table 1**, showing that SAIGE is the only mixed-model association method that is able to account for the unbalanced case-control ratios while remaining computationally practical for large data sets. To further evaluate the computational performance of SAIGE, we randomly sampled subsets from the 408,458 white British UK Biobank participants who are defined as either coronary artery disease (CAD) cases (31,355) or controls (377,103) based on the PheWAS Code 411[3,32,33] followed by benchmarking association tests using SAIGE and other existing methods on 200,000 genetic markers randomly selected out of the 71 million with imputation info $\geq$ 0.3. The non-genetic covariates sex, birth year, and principal components 1 to 4 were adjusted in all tests. The log10 of the memory usage and projected computation time for testing the full set of 71 million genetic variants are plotted against the sample size as shown in **Supplementary Figure 2** and **Supplementary Table 1**. Although SAIGE and BOLT-LMM have the same order of computational complexity (Table 1), SAIGE was slower than BOLT-LMM across all sample sizes (ex. 517 vs 360 CPU hours

when *N*=408,458). This is due to the fact that fitting logistic mixed model requires more iterative steps than linear mixed model, and applying SPA requires additional computation. SAIGE requires slightly less memory than BOLT-LMM (10 to 11 Gb when *N*=408,458) and the low memory usage makes both methods feasible for the large data set. In contrast, GMMAT and GEMMA requires substantially more computation time and memory usage. For example, when *N*=400,000, projected memory usages of both GMMAT and GEMMA are more than 600 Gb. The actual computation time and memory usage of association tests for the full UK Biobank data for CAD are given in **Table 1**. SAIGE required 517 CPU hours and 10.3 Gb memory to analyze 71 million variants that have imputation info $\geq$ 0.3 for 408,458 samples, which indicates that the analysis will be done in ~26 hours with 20 CPU cores.

Association analysis of binary traits in UK Biobank data

We applied SAIGE to several randomly selected binary traits defined by the PheWAS Codes (PheCode) of UK Biobank[3,32,33] and compared the association results with those obtained from the method based on linear mixed models, BOLT-LMM[25], and SAIGE without the saddlepoint approximation (SAIGE-NoSPA), which is asymptotically equivalent to GMMAT[26]. Due to computation and memory cost, the current GMMAT method cannot analyze the UK Biobank data. We restrict our analysis to markers directly genotyped or imputed by the Haplotype Reference Consortium (HRC)[37] panel due to quality control issues of non-HRC markers reported by the UK BioBank. Approximately 28 million markers with minor allele counts (MAC) ≥ 20 and imputation info score > 0.3 were used in the analysis. Among 408,961 white British participants in the UK Biobank, 132,179 have at least one up to the third degree relative among the genotyped individuals[32,33]. We used 93,511 high quality genotyped variants to construct the GRM. In the UK Biobank data, most binary phenotypes based on PheCodes (1,437 out of 1,663; 86.4%) have case-control ratio lower than 1:99 (**Supplementary Figure 3**) and would likely demonstrate problematic inflation of association test statistics without SPA.

Association results of three exemplary binary traits that have various case-control ratios are plotted in Manhattan plots shown in **Figure 1** and in the quantile-quantile (QQ) plots stratified by minor allele frequency (MAF) shown in **Figure 2**. The three binary traits are coronary artery disease (PheCode 411) with 31,355 cases and 377,103 controls (1:12), colorectal cancer (PheCode 153) with 4,562 cases and 382,756 controls (1:84) and thyroid cancer (PheCode 193) with 358 cases and 407,399 controls (1:1138). In the Manhattan plots in **Figure 1**, each locus that contains any variant with p-value < 5×10^{-8} is highlighted as blue or green to indicate whether this locus has been reported by previous studies or not. **Supplementary Table 2** presents the number of all significant loci and those that have not been previously reported by each method for each trait and **Supplementary Table 3** lists all significant loci identified by SAIGE.

Both Manhattan and QQ plots show BOLT-LMM and SAIGE-NoSPA have greatly inflated type I error rates. The inflation problem is more severe as case-control ratios become more unbalanced and the MAF of the tested variants decreases. The genomic inflation factors (λ) at the 0.001, 0.01 p-value percentiles are shown for several MAF categories in **Supplementary Table 4**. For the colorectal cancer GWAS which has case-control ratio 1:84, λ at the 0.001 p-value percentile is 1.68 and 1.71 for variants with MAF<0.01 by SAIGE-NoSPA and BOLT-LMM, while λ is 0.99 by SAIGE. The inflation is even more severe for the test results by SAIGE-NoSPA and BOLT-LMM for the thyroid cancer, which has case-control ratio 1:1138, with the λ at the 0.001 p-value percentile around 4 to 5 for variants with MAF<0.01 and all variants, respectively. With the unbalanced case-control ratio accounted for in SAIGE, the λ is again very close to 1.

Results for all ~1,600 PheCode-derived binary traits in 408,961 UK biobank white British European-

ancestry samples are currently being generated using SAIGE software and will be available in a public repository as soon as they have completed analysis (see below for URL).

Simulation Studies

We investigated the type I error control and power of two logistic mixed model approaches, SAIGE and GMMAT, and the linear mixed model method BOLT-LMM that computes mixed model association statistics under the infinitesimal and non-infinitesimal models through simulation studies. We followed the steps described on the Methods section to simulate genotypes for 1,000 families, each with 10 family members (N=10,000), based on the pedigree shown in **Supplementary Figure 4**.

Type I error rates

The type I error rates for SAIGE, SAIGE-NoSPA, GMMAT, and BOLT-LMM have been evaluated based on the association tests performed on 10^9 simulated genetic variants. The variants were simulated using the same MAF spectrum of the UK Biobank HRC imputation data with case-control ratio 1:99, 1:9, and 1:1. The empirical type I error rates at the $\alpha = 5x10^{-4}$ and $\alpha = 5x10^{-8}$ are shown in the **Supplementary Table 5**. Both SAIGE-NoSPA, GMMAT, and BOLT-LMM have greatly inflated type I error rates when the case-control ratios are moderately or extremely unbalanced and slightly deflated type I error rates when the case-control ratios are balanced. This is expected as previous studies have suggested inflation of the score tests in the presence of the unbalanced case-control ratios and deflation in balanced studies[29,38]. SAIGE has well corrected the inflation of GMMAT and BOLT-LMM when case-control ratios are unbalanced as well as the deflation when the case-control ratios are balanced. However, compared to the nominal α levels, the empirical type I error rates of SAIGE are slightly deflated.

To further investigate the type I error rates by MAF and case-control ratios, we carried out additional simulations. **Supplementary Figure 5** shows QQ plots of 1,000,000 rare variants (MAF = 0.005) with various case-control ratios (1:1, 1:9, and 1:99) and **Supplementary Figure 6** shows QQ plots of 1,000,000 variants with different MAF (0.005, 0.01, 0.05, 0.1 and 0.3) when case-control ratio was 1:99. Consistent to what has been observed in the real data study, GMMAT and SAIGE-NoSPA is more inflated for less frequent variants with more unbalanced case-control ratios. In contrast, SAIGE has successfully corrected this problem.

Power

Next we evaluated empirical power. Since power simulation requires re-estimating a variance component parameter for each variant to test, to reduce computational burden, we used SAIGE-NoSPA instead of the original GMMAT software. Due to the inflated type I error rates of GMMAT (and SAIGE-NoSPA), for a fair comparison, we estimated power at the test-specific empirical α levels that yield type I error rate $\alpha = 5x10^{-8}$ (**Supplementary Table 6**). **Supplementary Figure 7** shows the power curve by odds ratios for variants with MAF 0.05, 0.1 and 0.2. When the case-control ratio is balanced, the power of SAIGE, SAIGE-NoSPA and BOLT-LMM were nearly identical. For studies with moderately unbalanced case-control ratio (case:control=1:9), SAIGE has higher power than SAIGE-NoSPA and BOLT-LMM, which is due to very small empirical α for SAIGE-NoSPA and BOLT-LMM resulted from type I error inflation. The power gap is much larger when the case-control ratios are extremely unbalanced.

Overall simulation studies show that SAIGE can control type I error rates even when case-control ratios are extremely unbalanced and can be more powerful than GMMAT and BOLT-LMM. In contrast, GMMAT and BOLT-LMM suffer type I error inflation, and the inflation is especially severe with low MAF and unbalanced case-control ratios.

Code and data availability

SAIGE is implemented as an open-source R package available at https://github.com/weizhouUMICH/SAIGE/. The GWAS results for binary phenotypes in UK Biobank using SAIGE are currently available for public download at https://www.dropbox.com/sh/wuj4y8wsqjz78om/AAACfAJK54KtvnzSTAoaZTLma?dl=0
We will continue to populate the public download repository with results for all UK Biobank phenotypes (> 1,600) with the PheCodes[3] constructed based on ICD codes.

DISCUSSION

In this paper, we have presented a method to perform the association tests for binary traits in large cohorts in the presence of sample relatedness, which provides accurate p-value estimates for even extremely unbalanced case-control settings (with a prevalence < 0.1%). The dramatic decrease of the genotyping cost over the last decade allows more and more large biobanks to genotype all of their participants followed by genome-wide PheWAS, in which GWASs are performed for all thousands of diseases/conditions characterized based on EHR and/or survey questionnaires to identify genetic risk factors across different phenotypes[1–18]. Several challenges exist for PheWAS studies by large cohorts. Statistically, inflated type I error rates caused by unbalanced case-control ratios and sample relatedness need to be corrected. Computationally, most of existing mixed model association methods are not feasible for large sample sizes. Our method, SAIGE, uses logistic mixed model to account for the sample relatedness and applies the saddle point approximation (SPA) to correct the inflation caused by the unbalanced case-control ratio in the score tests based on logistic mixed models

SAIGE successfully corrects the inflation of type I error rates of low-frequency variants with binary traits that have unbalanced case-control ratios while also accounting for the relatedness among samples. Furthermore, our method uses several optimization strategies that are similar to those used by BOLT-LMM to improve its computational feasibility for large cohorts. For example, the preconditioned conjugate gradient algorithm is used to solve linear systems instead of the Cholesky decomposition method so that the time complexity for fitting the null logistic model is decreased from $O(N^3)$ to approximately $O(M_1N)$, where M_1 is the number of pruned markers used for estimating the genetic relationship matrix and the N is the sample size. Compared to large N, M_1 is usually small. For instance, in the UK Biobank[32,33], M_1 = 93,511 and N = 408,961 (white British participants).

There are several limitations in SAIGE. First, the time for algorithm convergence may vary among phenotypes and study samples given different heritability levels and sample relatedness. Second, SAIGE has been observed to be slightly conservative when case-control ratios are extremely unbalanced (**Supplementary Table 5**). Third, the accurate odds ratio estimation requires fitting the model under the alternative and is not computational efficient. Similar to several other mixed model methods[20,25,36], SAIGE estimates odds ratios for genetic markers using the parameter estimates from the null model. Fourth, SAIGE estimates the genetic relationship matrix using genome-wide genetic markers instead of using the

leave-one-chromosome-out (LOCO) scheme, which can avoid proximal contamination [23,25,39,40]. Last, SAIGE assumes that the effect sizes of genetic markers are normally distributed, which follows an infinitesimal architecture. With this assumption, SAIGE may sacrifice power to detect genetic signals whose genetic architecture is non-infinitesimal. In future direction, we will incorporate the LOCO scheme, which is straightforward based on the current model and method, and model non-infinitesimal architecture as needed to improve power. In addition, we will extend the current single variant test to gene- or region-based multiple variant test to improve power for identifying disease susceptibility rare variants.

With the emergence of large-scale biobank, PheWAS will be an important tool to identify genetic components of complex traits. Here we describe a scalable and accurate method, SAIGE, for the analysis of binary phenotypes in genome-wide PheWAS. Currently, SAIGE is the only available approach to adjust for both case-control imbalance and family relatedness, which are commonly observed in PheWAS datasets. In addition, the optimization approaches used in SAIGE make it scalable for the current largest (UK Biobank) and future much larger datasets. Through simulation and real data analysis, we have demonstrated that our method can efficiently analyze a dataset with 400,000 samples and adjust for type I error rates even when the case-control ratios are extremely unbalanced. Our method will provide an accurate and scalable solution for large scale PheWAS analysis and ultimately contribute to identify genetic mechanism of complex diseases.

METHODS

Generalized linear mixed model for binary traits

In a case-control study with sample size N, we denote the status of the *ith* individual using $y_i = 1$ or 0 for being a case or a control. Let the $1\times(1+p)$ vector X_i represent p covariates including the intercept and G_i represent the allele counts $(0, 1 \text{ or } 2)$ for the variant to test. The logistic mixed model can be written as

$$logit(\mu_i) = X_i\alpha + G_i\beta + b_i$$

where $\mu_i = P(y_i = 1 \mid X_i, G_i, b_i)$ is the probability for the *ith* individual being a case given the covariates and genotypes as well as the random effect, which is denoted as b_i. The random effect b_i is assumed to be distributed as $N(0, \tau\,\psi)$, where ψ is an $N \times N$ genetic relationship matrix (GRM) and τ is the additive genetic variance. The α is a $(1+p)\times 1$ coefficient vector of fixed effects and β is a coefficient of the genetic effect.

Estimate variance component and other model parameters(Step 1)

To fit the null model, $logit(\mu_{i0}) = X_i\alpha + b_i$, penalized quasi-likelihood (PQL) method[41] and the AI-REML algorithm[34] are used to iteratively estimate $(\hat{\tau}, \hat{\alpha}, \hat{b})$. At iteration *k*, let $(\hat{\tau}^{(k)}, \hat{\alpha}^{(k)}, \hat{b}^{(k)})$ be estimated $(\hat{\tau}, \hat{\alpha}, \hat{b})$, $\hat{\mu}_i^{(k)}$ the estimated mean of y_i, $\widehat{W}^{(k)} = diag[\hat{\mu}_i^{(k)}\left(1-\hat{\mu}_i^{(k)}\right)]$, and $\hat{\Sigma}^{(k)}$= $\{\widehat{W}^{(k)}\}^{-1} + \hat{\tau}^{(k)}\psi$ be an $n \times n$ matrix of the variance of working vector $\tilde{y}_i = X_i\alpha^{(k)} + b_i^{(k)} + (y_i - \hat{\mu}_i^{(k)})/\left\{\hat{\mu}_i^{(k)}\left(1-\hat{\mu}_i^{(k)}\right)\right\}$. To obtain log quasi-likelihood and average information at each iteration, the current GMMAT approach calculates the inverse of $\hat{\Sigma}^{(k)}$. Since it is computationally too expensive for large *N*, we use the preconditioned conjugate gradient (PCG)[30,31], which allows calculating quasi-likelihood and average information without calculating $\{\hat{\Sigma}^{(k)}\}^{-1}$ (See Supplementary for details). PCG is a numerical method to find solutions of linear system. It is particularly useful when the system is very large. BOLT-LMM[25] successfully used it to estimate variance component in linear mixed model.

A score test statistics for H_o: $\beta = 0$ is $T = G^T(Y - \hat{\mu}) = \tilde{G}^T(Y - \hat{\mu})$ where G and Y are $N \times 1$ genotype and phenotype vectors, respectively, and $\hat{\mu}$ is the estimated mean of Y under the null hypothesis, and $\tilde{G} = G - X(X^T\widehat{W}X)^{-1}X^T\widehat{W}G$ is the covariate adjusted genotype vector. The variance of T, Var(T) $= \tilde{G}^T P\tilde{G}$, where $\hat{P} = \hat{\Sigma}^{-1} - \hat{\Sigma}^{-1}X\left(X^T\hat{\Sigma}^{-1}X\right)^{-1}X^T\hat{\Sigma}^{-1}$. For each variant, given $\hat{P}$, calculation of Var(T) requires O(N^2) computation. In addition, since our approach does not calculate $\hat{\Sigma}^{-1}$, and hence $\hat{P}$, obtaining Var(T) requires applying PCG for each variant, which can be computationally very expensive. To reduce computation cost, we use the same approximation approach used in BOLT-LMM and GRAMMAR-GAMMAR[36], in which we estimate a variance of T with assuming that true random effect b is given, and then calculate ratio between these two variance. Suppose Var(T)* = $\tilde{G}^T\widehat{W}\tilde{G}$, which is a variance estimate of T assuming $\hat{b}$ is given. Let r = Var(T)/Var(T)* ratio of these two different types of variance estimates. In Supplementary materials, we have shown that the ratio is approximately constant for all variants. Using this fact, we can estimate r using a relatively small number of variants. In all the numerical studies in this paper, we used 30 variants to estimate r.

Score test with SPA (Step 2)

Suppose $\hat{r}$ is the estimated ratio (i.e. r) in Step 1. Now the variance adjusted test statistics is

$$T_{adj} = \frac{\tilde{G}^T(Y - \hat{\mu})}{\sqrt{\hat{r}\tilde{G}^T\widehat{W}\tilde{G}}},$$

which has mean zero and variance 1 under the null hypothesis. The computation of T_{adj} requires O(N) computation. The traditional score tests assume that T (and hence T_{adj}) asymptotically follows a Gaussian distribution under H_o: $\beta = 0$, which is using only the first two moments (mean and variance). When the case-control ratios are unbalanced and variants have low MAC, the underlying distribution of T_{adj} can be substantially different from Gaussian distribution. To obtain accurate p-values, we use Saddlepoint approximation method (SPA)[27–29], which approximates distribution using the entire cumulant generating function (CGF). A fast version of SPA (fastSPA)[29] has recently been developed and applied to PheWAS, and provides accurate p-values even when case-control ratios are extremely unbalanced (ex. case:control=1:600).

To apply fastSPA to T_{adj} we need to obtain CGF of T_{adj} first. To do this, we use the fact that given $\hat{b}$, T_{adj} is a weighted sum of independent Bernoulli random variables. The approximated cumulant generating function is

$$K(t;\ \hat{\pi}, c) = \sum_{i=1}^{N} \log\left(1 - \hat{\pi}_i + \hat{\pi}_i e^{ct\tilde{G}_i}\right) - ct\sum_{i=1}^{N} \tilde{G}_i\,\hat{\pi}_i$$

where the constant c=Var*(T)$^{-1/2}$. Let $K'(t)$ and $K''(t)$ are first and second derivatives of K with respect to t. To calculate the probability that $T_{adj} < q$, where q is an observed test statistic, we use the following formula[27,28,42]

$$pr\left(T_{adj} < q\right) \simeq F(q) = \Phi\left\{w + \frac{1}{w}\log\left(\frac{v}{w}\right)\right\},$$

where $w = sign(\hat{\zeta})\left[2\{\hat{\zeta}q - K(\hat{\zeta})\}\right]^{\frac{1}{2}}$, $v = \hat{\zeta}\{K''(\hat{\zeta})\}^{\frac{1}{2}}$ and $\hat{\zeta} = \hat{\zeta}(q)$ is the solution of the equation $K'(\hat{\zeta}) = q$. As fastSPA[29], we exploit the sparsity of genotype vector when MAF of variants are low. In

addition, since normal approximation works well when the test statistic is close to the mean, we use the normal distribution when the test statistic is within two standard deviation of the mean.

Data simulation

We carried out a series of simulations to evaluate and compare the performance of SAIGE to GMMAT. We randomly simulated a set of 1,000,000 base-pair "pseudo" sequences, in which variants are independent to each other. Alleles for each variant were randomly drawn from Binomial(n = 2, p = MAF). Then we performed the gene-dropping simulation[43] using these sequences as founder haplotypes that were propagated through the pedigree of 10 family members shown in **Supplementary Figure 4**. Binary phenotypes were generated from the following logistic mixed model

$$logit(\pi_{i0}) = \alpha_0 + b_i + X_1 + X_2 + G_i\beta$$

where G_i is the genotype value, β is the genetic log odds ratio, b_i is the random effect simulated from $N(0, \tau\,\psi)$ with $\tau = 1$. Two covariates, X_1 and X_2, were simulated from Bernoulli(0.5) and N(0,1), respectively. The intercept α_0 was determined by given prevalence (i.e. case-control ratios).

To evaluate the type I error rates at genome-wide $\alpha=5\times10^{-8}$, 10 million markers along with 100 sets of phenotypes with different random seeds for case-control ratios 1:99, 1:9, and 1:1 were simulated with $\beta = 0$. Given $\tau = 1$, the estimated heritability is 0.015, 0.092, and 0.17 for phenotypes with case-control ratios 1:99, 1:9, and 1:1, respectively[44]. Association tests were performed on the 10 million genetic markers for each of the 100 sets of phenotypes using SAIGE, GMMAT, and BOLT-LMM, therefore in total 10^9 tests were performed. To have a realistic MAF spectrum, MAFs were randomly sampled from the MAF spectrum in UK Biobank data (**Supplementary Figure 8**). Additional type I error simulations were carried out for five different MAFs (0.005, 0.01, 0.05, 0.1 and 0.3) to evaluate type I error rates by MAFs.

For the power simulation, phenotypes were generated under the alternative hypothesis $\beta \neq 0$. For each of the MAF 0.05 and 0.2, we simulated 1,000 datasets, and power was evaluated at test-specific empirical α, which yields nominal $\alpha=5\times10^{-8}$. The empirical α was estimated from the previous type I error simulations. As the same as type I error simulations, three different case-control ratios (1:99, 1:9, and 1:1) were considered.

Note that since we evaluated the empirical type I error rates and power based on genetic variants that were simulated independently, the LD Score regression[45] calibration and the leave-one-chromosome-out (LOCO) scheme were not applied in BOLT-LMM.

Phenotype definition in UK Biobank

We used a previously published scheme to defined disease-specific binary phenotypes by combining hospital ICD-9 and ICD-10 codes into heretical PheCodes, each representing a more or less specific disease group[3]. Cases were all study participants with the PheCode of interest and controls were all study participants without the PheCode of interest or any related PheCode. Gender checks were performed, so PheCodes specific for one gender could not mistakenly be assigned to the other gender.

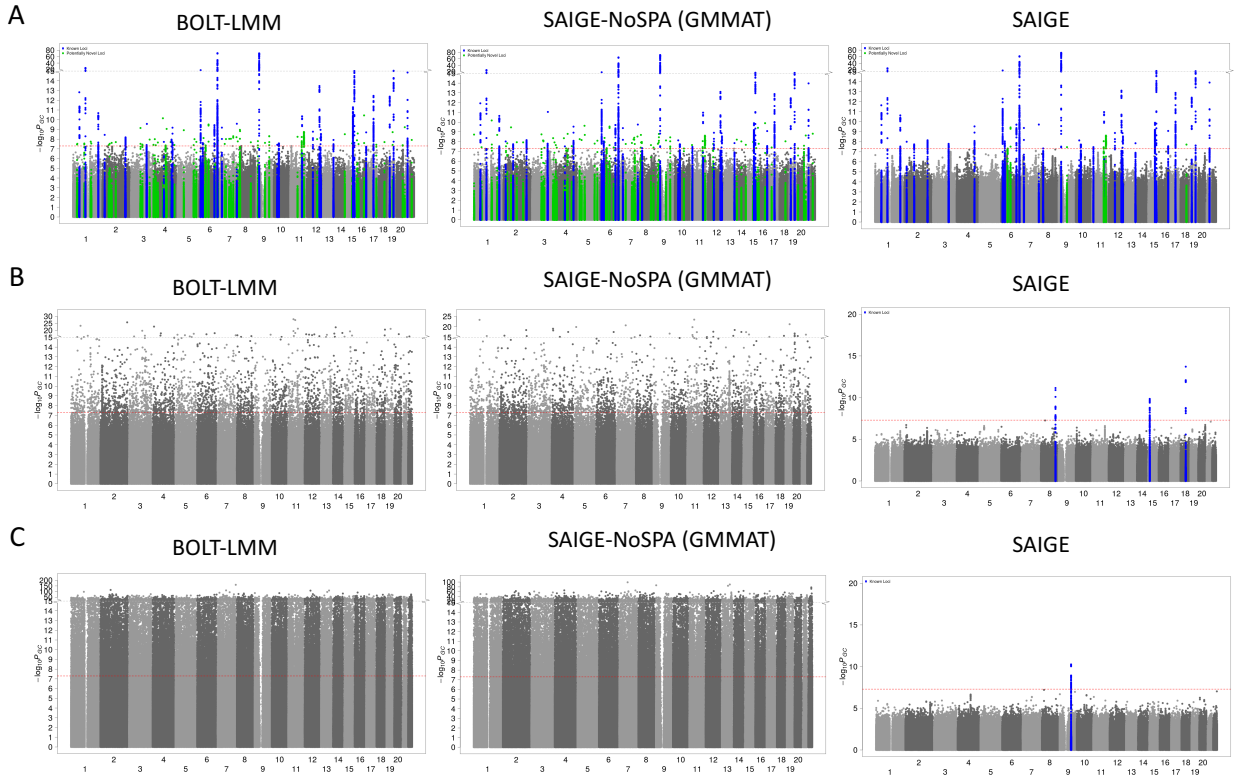

Figure 1. Manhattan plots of association p values resulting from SAIGE, SAIGE-NoSPA(asymptotically equivalent to GMMAT) and BOLT-LMM for A. coronary artery disease (PheCode 411, case:control = 1:12), B. colorectal cancer (PheCode 153, case:control = 1:84), and C. thyroid cancer (PheCode 193, case:control=1:1138). Blue: loci that have association p-value < $5x10^{-8}$, where the top hits are previously reported, Green: loci that have association p-value < $5x10^{-8}$ and have not been reported before. Since results from SAIGE-noSPA and BOLT-LMM contain many false positive signals for colorectal cancer and thyroid cancer, the significant loci are not highlighted.

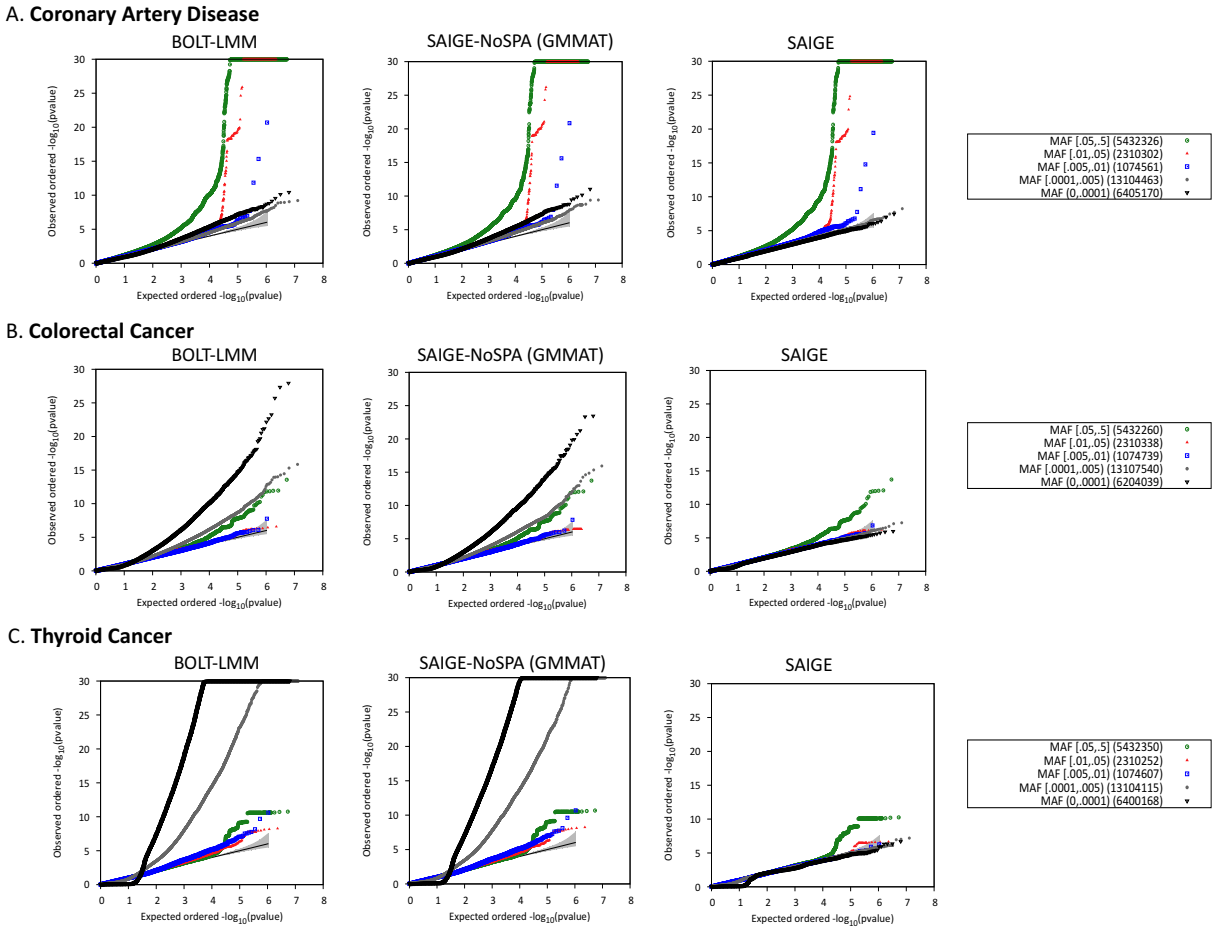

Figure 2. Quantile-quantile plots of association p-values resulting from SAIGE, SAIGE-NoSPA (asymptotically equivalent to GMMAT) and BOLT-LMM (non-infinitesimal mixed model association test p-value) A. coronary artery disease (PheCode 411, case: control = 1:12), B. colorectal cancer (PheCode 153, case: control = 1:84), and C. thyroid cancer (PheCode 193, case: control=1:1138).

Table 1. Comparison of different methods for GWAS with mixed effect models

		Method Features					Algorithm Complexity				Benchmarks for UK Biobank Data Coronary Artery Disease (PheCode 411)	
		Does not require pre-computed genetic relationship matrix	Feasible for large sample sizes	Developed for binary traits	Accounts for unbalanced case-control ratio	Tests quantitative traits	Time complexity		Memory usage (Gbyte)		Time CPU hrs	Memory
							Step 1	Step 2	Step 1	Step 2		
Logistic mixed model	SAIGE	✓	✓	✓	✓	✓	$O(PM_1N^{1.5})$ *	$O(MN)$	$M_1N/4$	N	517	10.3G
	GMMAT			✓		✓	$O(PN^3)$	$O(MN^2)$	FN^2	FN^2	NA	NA
Linear mixed model	BOLT-LMM	✓	✓			✓	$O(PM_1N^{1.5})$ *	$O(MN)$	$M_1N/4$	N	360	10.9G
	GEMMA					✓	$O(N^3)$	$O(MN^2)$	FN^2	FN^2	NA	NA

N: number of samples
P: number of iterations required to reach convergence
M_1: number of markers used to construct the kinship matrix;
M: total number of markers to be tested
F: Byte for floating number
* Number of iterations in PCG is assumed as $O(N^{0.5})$[25]

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