Frequentist tests of association for imputed genotypes

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Abstract

Servin and Matthews [17] proposed looking for associations between phenotypes and both typed and untyped SNPs, by using a reference panel to infer the alleles of the untyped SNPs. Since then, a number of GWA studies have reported p-values for both typed and untyped SNPs. However, results of Almeida *et al*[1] indicate that using imputed genotype data can lead to increased type I error. We discuss possible causes of inflated type I error from using imputed data and propose methodology designed for conducting GWAS studies with imputed genotypes.

1 Introduction

A primary focus of genetics is the identification of the genetic variants causing variation in a particular phenotype of interest, such as height, susceptibility to diabetes, or drug sensitivity. The earliest discoveries of such genetic variants were made by linkage analyses–studies which compared genotypes within the same family. However, such studies failed to discover variants accounting for the genetic basis of complex diseases such as diabetes. This led geneticists to suspect that the genetic component of complex diseases were distributed among many genes with small effects on disease susceptibility. As Risch and Merikangas noted in their seminal paper [16], it would be very difficult to discover such small-effect variants with linkage studies; however, it could be much more feasible to discover such variants by carrying out association studies on unrelated individuals.

Indeed, Risch and Merikangas anticipated the introduction of the genome-wide association (GWA) study. In a GWA study, the genotypes of possibly unrelated case and control subjects are compared to find associations between phenotypes and genetic variants: single-nucleotide polymorphisms (SNPs) or less commonly, copy-number variations [4]. Such associations can help narrow the search for the true causative variants: due to the strong correlation between variants located close to one another on a chromosome (linkage disequilibrium), a causative variant will induce associations between nearby variants and the phenotype. That said, not all putative associations found in GWA studies are necessarily induced by linkage disequilibrium with a causative variant: many could be caused by population stratification, sampling biases, or false positives [14]. Despite these shortcomings, researchers have suceeded in using GWA studies to uncover interesting genetic variants involved in important diseases, including type I diabetes and Crohn's disease [22].

A major component to the cost-effectiveness of GWA studies is the fact that interesting discoveries can be made by sequencing a relatively small subset of the subjects' genomes. Thus GWA studies typicially rely on tag-SNP chips which allow them to cheaply sequence on the order of 300,000 sites in the genome. These tag-SNP sites are chosen to exploit the block patterns found in human genetic variation. As discovered in the HapMap project, 5-15 kilobase blocks of alleles, or haplotype blocks, tend to be coinherited– and the diversity of haplotype blocks in a given population can be quite low [9]. Meanwhile, the tag SNPs are chosen to optimally distinguish common haplotypes for each block. Therefore, causative variants included in a common haplotype will likely induce an association between the tag SNPs chosen for that block.

Interestingly, though, due to the availability of population-level SNP and whole-genome data provided by sequencing efforts such as HapMap and the 1000 genomes project, it is possible to accurately impute many additional SNPs of a subject from their genotyped SNPs. Servin and Matthews [17] proposed a statistical framework to find associations between the phenotypes and the imputed SNPs in addition to the typed SNPs, which could possibly lead to more discoveries. Almeida *et al.* [1] investigated the reliability of results obtained from imputed SNPs. Using case-control data from a type I diabetes study, they compared p-values obtained from a set of genotyped SNPs with p-values obtained from imputed values from the same SNPs. They found that less than half of the imputation-based p-values below the threshold of 10^{-5} had corresponding genotype-based p-values below the threshold, and concluded that the use of imputed SNPs leads to an inflated type I error rate. Nevertheless, a number of recent meta-analyses [6][11] have used imputed SNPs.

Since Sevin and Matthews first proposed the analysis of imputed SNPs, a number of algorithms for genotype imputation have been developed. The most recent algorithms can automatically find boundaries of haplotype blocks, as well as automatically perform phasing on both reference panel data and case/control data. Marchini and Howie [13] provide a detailed review of the most popular algorithms.

1.1 Statistical Evaluation

An extensive body of frequentist statistical literature applies to GWA studies using only genotyped SNPs [24]. Chiefly, there exist two major methodologies for multiple testing which can be applied to interpret the p-values obtained from genotyped data: the Bonferroni procedure, which controls the the family-wise error rate (FWER) and the Benjamini-Hochberg (BH) procedure, which controls the false discovery rate (FDR) [20]. But while the majority of GWA studies do use marginal association tests for the individual SNPs, whether genotyped or imputed, most do not bother to apply either multiple testing procedure, instead reporting p-values below a prespecified threshold, e.g. 10^{-5}.

A major reason for this practice is that the unmodified Bonferonni and BH procedure are overly severe when dealing with large numbers of highly correlated test statistics. Lin [12] and Dudbridge [5] have suggested using permutation-test based modifications to the Bonferonni or BH procedure which ameliorate the effect of high correlations on statistical power. Storey and Tibshriani [20] also propose a permutation-test based procedure to control false discovery rate, which is theoretically conservative under "weak dependence" conditions. We go into more detail about these modified Bonferonni and BH procedures in Section 2.

But moreover, as Ziegler *et al* note, neither the control of FWER nor FDR "overcome the fundamental problem that formal statistical testing often is not the primary aim in a GWA...[which is] to know whether a SNP is worthy of further investigation..."

Indeed, even supposing that such frequentist procedures are theoretically effective for detecting associations, there is the additional issue that such theoretical properties depend on a “sparse effects” assumption in which all uninteresting SNPs have zero association with the phenotype. Yet this assumption is most definitely violated in practice: recall that any SNPs in linkage disequilibrium with a causative variant would have association with the phenotype will formally be considered non-null features. Thus neither frequentist procedure is guaranteed to control the number of such SNPs reported. This is a minor issue if such SNPs are close to the causative SNP, but Reich *et al* [15] find that within the European population regions of detectible linkage disequilibrium routinely extend to 160 kilobases around an SNP. Given the effort required to hunt for a causative variant in a 5 kb region, one would surely consider an SNP which is 160 kb away from a causative variant to be uninteresting; yet the established framework for multiple testing makes no special provision to exclude such hits.

For these and other reasons, many statisticians have proposed abandoning single-marker analysis altogether, advocating regression [7] or other machine learning-based approaches, or Bayesian inference [17]. Such alternative approaches generally have a shorter history than the body of frequentist work: significance testing for high-dimensional regression is currently an extremely active area of research, with many new approaches proposed within the last two years [3][10][21]. Bayesian modelling approaches, though already quite complicated, still have enormous room to grow, including incorporation of population structure [18].

While both acknowledging the known disadvantages of single-marker-based frequentist analysis and the promise of alternative methodologies to GWA studies, we will consider the possible use of the multiple-testing framework on both genotyped and imputed SNP data in the rest of the paper.

As we mentioned, a major shortcoming of the current multiple testing framework is the fact that it fails to formally discriminate between SNPs strongly associated with the phenotype and SNPs very weakly associated with the phenotype. A second major shortcoming of the current body of frequentist methodology is the lack of a framework for dealing with imputed data.

However, we feel that both of these shortcomings can be overcome, and in this paper, we will discuss possible ways to do so. If, indeed, a satisfactory frequentist testing procedure can be developed which overcomes these shortcomings while maintaining an adequate power, the use of such a procedure in GWA studies would yield an important benefit: a metric to gauge the reliability of putative associations. Researchers would be able to use these frequentist measures to inform their decisions on whether or not to follow up on the SNPs found by a particular study or meta-analysis.

The first shortcoming of the frequentist approach, which is its failing to distinguish between strong and weak effects, can be addressed in a number of ways. One approach, proposed by Wager [23] in an unpublished paper, is to define an "irrelevance coefficient" which penalizes features with small effects, and to control the average irrelevance coefficient of the reported features analogously to how the BH procedure controls the average proportion of false discoveries within the reported features. For reasons of convenience, we take a simpler approach, which is to redefine a null feature to be one with a possibly nonzero effect which falls below a threshold. The effect of this on existing procedures is to modify the method of computing p-values.

Under this new perspective, a Type I error corresponds to misclassifying a weak effect as a strong effect. This corresponds more closely to scientific realities, in which the discovery of a weakly associated SNP is not much preferable to the discovery of an unassociated SNP. Existing procedures will continue to control the Type I error given the modified method of computing p-values when using genotyped data. However, an increased Type I error rate (in the form of increased FWER or increased FDR) can still occur if the Bonferroni or BH procedure are used on imptued data.

In this project, we explore population-level phenomenona which could lead to failures of the Bonferroni or BH method are naively applied to imputed SNPs; additionally, we suggest a novel statistical procedure designed specifically for carrying out hypothesis testing with imputed data, which continues to incorporate the threshold-based definition of a null hypothesis.

2 Testing of associations in genotyped data

Let $\mathcal{S} = \{s_1, \ldots, s_M\}$ be a set of biallelic SNPs of interest, and let us fix a reference haplotype. Let $(Z, X^{(1)}, X^{(2)})$ be random variables representing a phenotype Z taking values $\{0, 1\}$ and two haplotypes $X^{(1)}, X^{(2)}$ for individuals in a population, where $X^{i,(j)}$ is the value of the SNP s_i in the jth haplotype; $X^{i,(j)} = 0$ if the SNP has the same allele as the reference.

We obtain data for L cases and L controls in the following manner. We sample the cases as iid draws from the population conditional on $Z = 1$, and the controls as iid draws conditional on $Z = 0$. Given this sampling, we obtain genotypes $X_1^{(1)}, X_2^{(2)}, \ldots, X_L^{(1)}, X_L^{(1)}$ for controls and $X_{L+1}^{(1)}, X_{L+1}^{(2)}, \ldots, X_{2L}^{(1)}, X_{2L}^{(2)}$

for cases. These genotypes could variant calling or phasing errors, but we will assume that such errors affect cases and controls in the same way.

Given this data, we compute for each SNP s_i the total minor allele count for cases T_i and the total minor allele count for controls U_i,

$$U_i = \sum_{k=1}^{L_0} X_k^{i,(1)} + X_k^{i,(2)}$$

$$T_i = \sum_{k=L_0+1}^{L} X_k^{i,(1)} + X_k^{i,(2)}$$

Given this, we compute the empirical log-odds ratio $\hat{\theta}_i$ for the ith SNP:

$$\hat{\theta}_i = \log(T_i) - \log(2L_1 - T_i) + \log(2L_0 - U_i) - \log(U_i),$$

an estimate of the true log-odds ratio θ_i, and the empirical variance[**?**] of the estimate:

$$V_i = \frac{1}{U_i} + \frac{1}{2L_0 - U_i} + \frac{1}{T_i} + \frac{1}{2L_1 - T_i}$$

Now we set a threshold τ for "small-effect" SNPs, e.g. $\tau = 0.05$. For low τ, SNPs with odds ratios between approximately $1 - \tau$ and $1 + \tau$ will being classified as "small-effect". Setting $\tau = 0$ recovers the standard paradigm of hypothesis testing where null SNPs have odds ratios of exactly 1. A type I error corresponds to reporting such a "small-effect" SNP.

Given only a specification of τ, we can compute the raw p-values to be used in the multiple testing procedure as

$$P_i = 2 - 2\Phi\left(\frac{(\hat{\theta}_i - \tau)_+}{\sqrt{V_i}}\right)$$

where $(x)_+ = xI(x > 0)$ and Φ is the normal cumulative distribution function.

After obtaining the raw p-values, any of the multiple testing procedures [12] [5] [20] can be applied to assess the statistical significance of the findings.

In the paper we will mainly focus on the use of the original Benjamini-Hochberg procedure [2]:

BH procedure

1. Fix a false discovery rate threshold q
2. Given p-values $p_1, \ldots, p_M$, sort them in ascending order:

$$p_{(1)} \leq p_{(2)} \leq \cdots \leq p_{(M)}$$

3. Compute $t_1, \ldots, t_M$ by $t_i = \frac{M}{i} p_{(i)}$
4. Let j be the largest index such that $t_j \leq q$
5. Let $p_{c_1}, \ldots, p_{c_j}$ be the j smallest p-values. Report the SNPs $s_{c_1}, \ldots, s_{c_j}$.

Let V be the number of null SNPs reported, and R the total number of null SNPs reported. Then the false discovery rate (FDR) is $\mathbf{E}[V/R]$, where the expectation is taken over the sampling distribution of the data. The BH procedure has the property of controls the false discovery rate, i.e.

$$\mathbf{E}\left[\frac{V}{R}\right] \leq q$$

3 Naive Testing of Imputed Data

Now we consider what could go wrong if the procedure outlined in section 2 is applied to imputed data. We modify the setup of section 1 by supposing that a reference panel is available for the full set of SNPs $\mathcal{S}$, but that only a subset $\mathcal{S}_{tag}$ of the SNPs are observed for the case and control data: without loss of generality, let $s_1, \ldots, s_N$ be the observed SNPs. Here we are assuming that the observed genotypes are correctly phased: again, this is an assumption made for the sake of discussion, and in reality there would be additional issues due to the uncertainty of phasing.

The reference panel gives rise to an imputation rule $\hat{Q}$, a function of a haplotype containing observed SNPs. While results may differ depending on the particular imputation rule, we claim that the most widely used approaches [13] can be interpreted as methods to estimate the conditional joint distribution of a haplotype in the reference population given its observed elements

$$\hat{Q}(X^{(j)}|X^{1,(j)}, \ldots, X^{N,(j)}) \approx \Pr[X^{(j)}|X^{1,(j)}, \ldots, X^{N,(j)}]$$

In fact, an idealized imputation rule would replicate the distribution precisely, while a “good” imputation rule would differ slightly from the true conditional distribution. However, as we will demonstrate, even an idealized imputation rule can lead to type I error due to differences between the reference population and the case or control populations.

Given such an imputation rule, Guan and Stephens [8] describe procedures to generate either Bayes factors or likelihood-ratio-based test statistics which take into account the uncertainty of the imputation. They also conclude that in the Bayesian framework, similar performance can be obtained by using the posterior mean of the conditional distribution. However, recent studies [6][11] suggest that the current practice in meta-analyses is to directly impute SNPs, apply quality filters, and then apply standard association tests as the imputed values were real data. Hence in the following, we will consider using the posterior mean imputed values, and then naively applying the procedure in section 2: we feel that such a procedure is only a slight modification of current practice.

To elaborate, given the imputation rule $\hat{Q}$, interpreted as an estimate of the conditional probability distribution of the untyped SNPs in the reference population, we produce imputed haplotypes $\tilde{X}_i^{N+1,(j)}, \ldots, \tilde{X}_i^{M,(j)}$ for each individual i and haloptype j, by

$$\tilde{X}_i^{k,(j)} = \hat{Q}(X^{k,(j)} = 1 | X_i^{1,(j)}, \ldots, X_i^{N,(j)})$$

Then the procedure in section 1 is applied, as if the imputed haplotype $\tilde{X}^{(j)}$ was originally observed.

The question we now consider is: under what conditions will this naive protocol lead to an increased type I error, in the form of either increased FWER or increased FDR?

Remark. Recall that in this context, an increased type I error means misclassifying an SNP with small effect (that is, with absolute log-odds less than τ) as an SNP with non-small effect. If $\tau = 0$, corresponding to the classical definition of null hypothesis, the question has a trivial answer, since for any SNP associated with the phenotype, all SNPs in linkage disequilibrium with the SNP will also be associated with the phenotype, and hence be considered non-null. The only source of increased type I errors is if an SNP independent of the phenotype has a different imputed-value distribution between cases and controls. Yet, in order for the SNP to have a different imputed-value distribution, there must exist at least one other SNP must be considered to be in LD with the null SNP (according to the imputation rule $\hat{Q}$) which has an association with the phenotype. Yet, in reality, it is not possible for there to exist any SNP associated to the null SNP which is associated with the phenotype. **Therefore, under the classical definition of null hypothesis, increased type I error is only possible when the imputation rule $\hat{Q}$ erroneously attributes dependence between two independent SNPs.** But some population-genetics assumptions would make the problem of failing to capture independence relationships a practical non-issue. It is generally realistic to assume that the reference population contains the case and control populations as subsets, and furthermore, that the reference population is more "diverse." In particular, we would assume that any pair of SNPs which are independent in the case or control population are independent in the reference population. Supposing this is the case, then the risk for increased type I error is mitigated if the imputation algorithm captures the haplotype block structure accurately: a task which many algorithms can accomplish fairly well [19]. It is important to note, though, that for $\tau > 0$, the problem of controlling type I error becomes much more challenging, and even an idealized imputation rule can lead to increased type I error.

Given the threshold-based definition of null SNPs, we note the following possible causes of increased type I error.

1. *Miscalled SNPs.* Miscalled variants in the typed SNPs could lead to incorrectly imputed posterior means in the untyped SNPs. Almeida *et al* [1] noted that filtering for variant call quality improved the agreement between imputed p-values and p-values obtained from genotyped data.

2. *Inaccuracies in imputation rule relative to reference.* A low sample size for the reference population limits the accuracy of the derived imputation rule, especially if haplotypes common in either the case or control but rare in the reference population are not included in the reference panel. If two SNPs are weakly asssociated in $\hat{Q}$ but strongly associated in the reference population, this not lead to increased type I errors, since weak associations in the imputation rule tend to lead to imputed allele sums being similar in cases vs controls. Increased type I error could more likely occur if two SNPs strongly associated in $\hat{Q}$ are in reality weakly associated. Suppose the imputation rule contains a strong association between a typed SNP s_s strongly associated with a phenotype and an SNP s_w very weakly associated with the phenotype, but in the control and/or case population, s_s is only weakly associated with s_w. Then the difference in case counts of s_s and control counts of

s_s would lead to a significant difference in imputed sums of s_w in the cases vs controls, when in reality the ratios of sums in cases vs controls is much closer to 1.

3. *Mismatch between the reference and study population.* Even supposing the imputation rule is quite faithful to the reference population, there may be a mismatch between the reference population and study population from which cases and controls are drawn. If the reference population contains a strong association between a typed SNP s_s strongly associated with a phenotype and an SNP s_w very weakly associated with the phenotype, but in the control and/or case population, s_s is only weakly associated with s_w, this could lead to increased type I error, for the same reasons as mentioned immediately above.

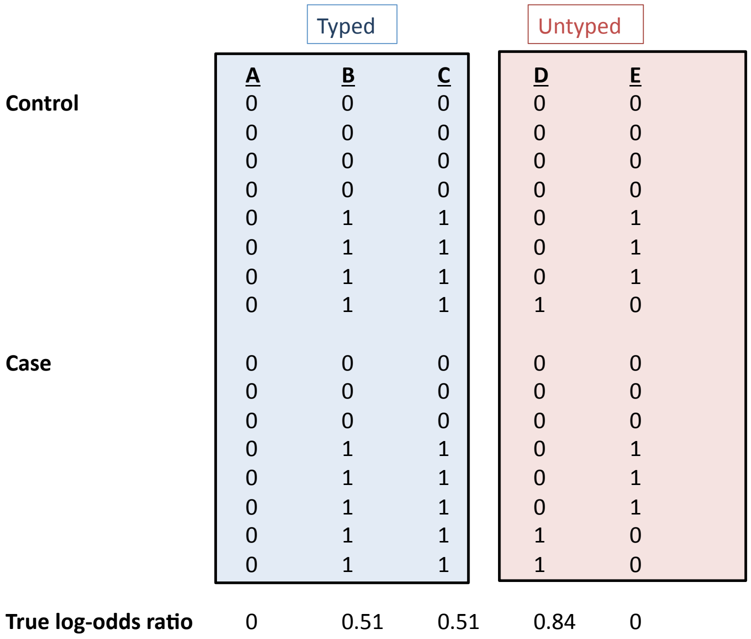
4. *Population-level causes.* Even with a reference panel derived from the same population as the controls, and an idealized imputation rule, increased type I errors could still occur. See next subsection.

Before we move on for a more detailed discussion of population-level causes, we remark on a counter-intuitive aspect of the problem. Note carefully that an inadequate sample size does *not* appear on the list for possible reasons of increased type I error. This is because frequentist procedures automatically account for the increased variance due to low sample size–that is, until the sample size begins to cause violations to crucial assumptions such as normality of the test statistics. However, since the procedures do *not* account for possible errors due to imputation, type I error for imputed SNPs may, indeed, increase dramatically as sample sizes increase. This is because the only way for a type I error to occur is for the erroneous signals in the imputed SNPs to gain sufficient strength to overcome the p-value threshold used.

3.1 Population-level causes of increased type I error

Suppose that the reference and control population are the same, and the imputation rule $\hat{Q}$ captures the true conditional distribution of haplotypes in the control population.

We provide a scenario to illustrate how a type I error can still occur. Consider a haplotype block with 5 SNPs, labeled A, B, C, D, E (Fig .1) Only B, C, and D have strong associations with the phenotype. Note that while in the figure, E appears to have an odds ratio of zero, this is only due to the discreteness of the example: realistically, in a large population of cases and controls, it would have true odds ratio close but not equal to zero. A reference panel provides the joint distribution of all five SNPs. However, only A, B, and C are genotyped for cases and controls. The distribution of haplotypes within the haplotype block for the control population is very similar to the distribution for the reference population.

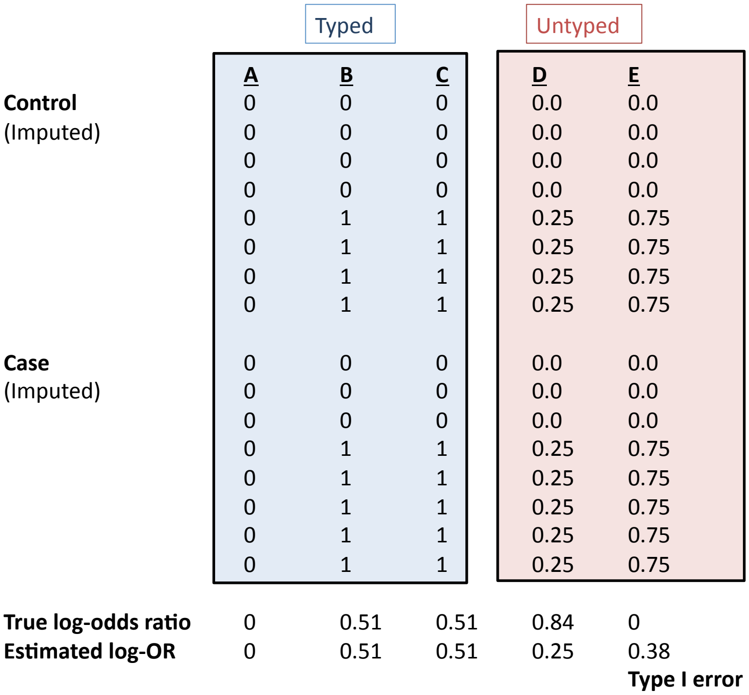


	Typed			Untyped	
	A	**B**	**C**	**D**	**E**
Control	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	1	1	0	1
	0	1	1	0	1
	0	1	1	0	1
	0	1	1	1	0
Case	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	1	1	0	1
	0	1	1	0	1
	0	1	1	0	1
	0	1	1	1	0
	0	1	1	1	0
True log-odds ratio	0	0.51	0.51	0.84	0

Figure 1: True distribution of case and control population

Given a sufficiently large sample size, the proportions of the haplotypes in the case and control samples will closely match the population-level distributions shown in Fig 1. But when this is the case, the estimated log-odds ratio for E will be much larger than than the true log-odds ratio, leading to a type I error: this is illustrated in Figure 2.

We can generalize this simple example to understand a class of patterns which would give rise to type I errors for imputed data *which are intrinsic to population-level distributions of haplotypes for the cases and controls.* In



	Typed			Untyped	
	A	**B**	**C**	**D**	**E**
Control (Imputed)	0	0	0	0.0	0.0
	0	0	0	0.0	0.0
	0	0	0	0.0	0.0
	0	0	0	0.0	0.0
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
Case (Imputed)	0	0	0	0.0	0.0
	0	0	0	0.0	0.0
	0	0	0	0.0	0.0
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
	0	1	1	0.25	0.75
True log-odds ratio	0	0.51	0.51	0.84	0
Estimated log-OR	0	0.51	0.51	0.25	0.38 **Type I error**

Figure 2: Estimated log-odds ratios of imputed SNPs

the example, there are three haplotypes, which we call α, β, γ:

$$\begin{aligned} \alpha &:(00000) \\ \beta &:(01110) \\ \gamma &:(01101) \end{aligned}$$

Note that crucial to this example is the fact that β and γ are indistinguishable given the typed SNPs, and that γ is much more rare than β in the control population. Yet, γ is enriched in the case population, while β is not. A possible mechanism is that the minor variant for SNP E, which is unique to haplotype γ, is a causative variant for the phenotype. The final ingredient is the fact that the minor variant for SNP D is unique to β. Otherwise, if SNP D was also present in haplotype γ, while the SNP may not be causative, it would still be associated with the phenotype, and still technically count as a true positive.

We generalize the example to a class of population-level patterns leading

to type I errors:
Pattern I

1. There exist two haplotypes β, γ for a given haplotype block, which are indistinguishable given the typed SNPs.

2. The haplotype γ is enriched in either the case or control population, while β is enriched in neither the cases nor controls.

3. The haplotype β contains minor variants not present in γ, nor any other haplotype which is enriched in the case population.

4. The haplotype β is relatively common in the reference population, while γ is rare.

This effect can persist even if there exist other haplotypes δ, ϵ etc. possibly also containing SNPs associated with the phenotype, as long as the total increase in rare haplotypes in the case population relative to the reference population remains small relative to τ.

We suggest a plausible geneological mechanisms for how such haplotypes β, γ could arise within a haplotype block in Figure 3. Initially, a common ancestor ζ of both β and γ propagates. Both the β and γ haplotypes emerge; but somehow, the β haplotype ends up occupying a large proportion of the haplotypes descended from ζ. Crucially, the mutation associated with the γ haplotype does not reappear, in a large fraction, in any of the β haplotypes. Such a process could occur, in parallel, over all of the haplotype blocks in the genome.

Still, the actual impact on type I error rates depends on the relative fraction of haplotype blocks containing such β,γ haplotypes. It would be interesting, and perhaps informative, to assess the likelihood of Pattern I arising in a population genetics framework, in relation to the number of typed SNPs. If the prospect is relatively unlikely given a decent number of typed SNPs this would indicate less need for caution in testing imputed data. All the same, we show in the next section that it is possible to get guaranteed type I error control for imputed data.

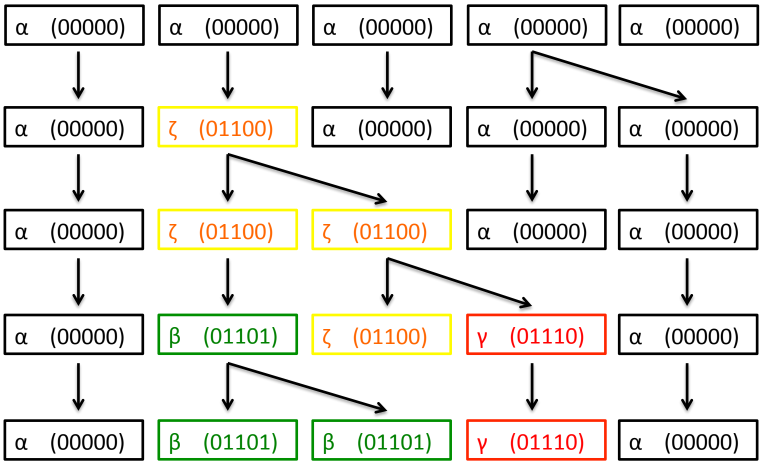


Figure 3: Possible geneological mechanism for Pattern I

4 A nonparametric method for testing associations in imputed data

The fundamental problem with naively applying a multiple testing method to imputed data is that the implicit assumption that the imputation rule captures the dependency structure of the cases and controls. When this assumption is violated, there is no guarantee of type I control. However, we can test the validity of this key assumption by paying an extra price: genotyping a larger set of SNPs on a *validation sample*.

Using this idea, we develop a method for testing associations in imputed data which can deal with all of the possible causes of failure listed in section 3: miscalled SNPs, inaccurate imputation rule, mismatch between reference and control, and population-level causes.

4.1 Procedure

Let us continue with the setup of section 3. This time, in addition to collecting a subset $\mathcal{S}_{tag}$ on L individuals, we will sequence an additional L_{val} individuals. From this validation sample, we genotype both $\mathcal{S}_{tag}$ and an additional set of validation SNPs $\mathcal{S}_{val}$. The set of validation SNPs is randomly selected from the set of SNPs of interest, minus the tag SNPs. That is, letting N_v be the number of validation SNPs,

$$\mathcal{S}_{val} = \{s_{I_1}, \ldots, s_{I_{N_v}}\}$$

where $I_1, \ldots, I_{N_v}$ are selected uniformly at random without replacement from $N+1, \ldots, M$.

Our data is as follows:

- A reference panel, which yields an imputation rule $\hat{Q}$.
- Phased genotypes for $\mathcal{S}_{tag}$ from L cases and L controls

  $$X_i^{1,(1)}, X_i^{1,(1)}, \ldots, X_i^{N,(1)}, X_i^{N,(2)}$$

  with $i = 1, \ldots, L$ for controls and $i = L+1, \ldots, 2L$ for cases.
- Phased genotypes for $\mathcal{S}_{tag}$ and randomly selected validation SNPs $\mathcal{S}_{val}$ from L_{val} cases and L_{val} controls

  $$X_i^{1,(1)}, X_i^{1,(1)}, \ldots, X_i^{N,(1)}, X_i^{N,(2)}, X_i^{I_1,(1)}, X_i^{I_1,(2)} \ldots, X_i^{I_{N_v},(1)}, X_i^{I_{N_v},(2)}$$

- Imputed genotypes for L cases and L controls, $\tilde{X}_i^{(j)}$ for $i = 1, \ldots, 2L$ and $j = 1, 2$.

Our procedure involves two control parameters, α_1, α_2, in addition to the discovery threshold τ. We will make use of the assumption that the true number of null SNPs is greater than the true number of alternative SNPs.

Procedure for Imputed Genotypes

1. Conduct the standard testing procedure for the tag SNPs on combined sample of $L + L_{val}$ cases and $L + L_{val}$ controls as a separate analysis

2. Obtain p values for validation SNPs

$$\mathcal{P}^{val} = \{p^{val}_{I_1}, \ldots, p^{val}_{I_{N_v}}\}$$

   using the formula presented in section 2

3. *(BH procedure for validation data)* From the validation data, we will obtain a set of *presumed null* SNPs. Apply original BH procedure[2] with $q = 0.5$ to $\mathcal{P}^{val}$. Define the set of presumed nulls

$$\mathcal{S}_{prenull} = s_{J_1}, \ldots, s_{J_A}$$

   as the subset of validation SNPs *accepted* by the BH procedure.

4. Obtain imputed p values for the L cases and L controls $\tilde{p}_1, \ldots, \tilde{p}_M$.

5. The set of imputed p values for $\mathcal{S}_{prenull}$

$$\tilde{\mathcal{P}}_{prenull} = \{\tilde{p}_{J_1}, \ldots, \tilde{p}_{J_A}\}$$

   will be used to estimate the distribution of the imputed p values of the null SNPs.

6. Given $\tilde{\mathcal{P}}_{prenull}$ and parameter α_1, obtain a set of transformed imputed p-values for the untyped SNPs $\mathcal{S}_{imputed} = \mathcal{S} \setminus \mathcal{S}_{tag}$,

$$\hat{p}_i = \hat{L}(\tilde{p}_i)$$

   for $i = N+1, \ldots, M$ (details in next section).

7. *(BH procedure for main data)* Apply the original BH procedure with $q = \alpha_2$ to the transformed p values $\hat{\mathcal{P}} = \{\hat{p}_{N+1}, \ldots, \hat{p}_M\}$. Report the SNPs rejected by the BH procedure for the main data.

This procedure will control the False Discovery Rate at level α, where α is a function of α_1, α_2, L, L_{val}, and N_v and $M - N$, but independent of the data generating mechanism. (details in following section).

4.2 Rationale

We outline the strategy behind our procedure.

Given the complete data for all SNPs in $\mathcal{S}_{imputed}$, we would have a distribution of p-values for non-screening case and controls, $p_{N+1}, \ldots, p_M$. However, we have p-values for imputed SNPs derived from the imputation rule $\hat{Q}$ for L case and L controls, $\tilde{p}_{N+1}, \ldots, \tilde{p}_M$.

Let $I_0 = \{\iota_1, \ldots, \iota_{M_0}\}$ be the true set of null SNPs in $\mathcal{S}_{impute}$. If the marginal distribution of $\tilde{p}_{\iota_i}$ were uniform, or stochastically dominated the uniform distribution, then the existing frequentist approaches would correctly control the type I error. However, the problem is that due to errors induced by imputation, the marginal distribution of $\tilde{p}_{\iota_i}$ may not stochastically dominate the uniform distribution. The crux of our proposed solution is to *estimate* the aggregate distribution of $\tilde{p}_{\iota_i}$ across all null SNPs, and then use this estimate to obtain transformed p-values $\hat{p}_{N+1}, \ldots, \hat{p}_M$ so that with a high probability, the transformed p-values of the nulls $\hat{p}_{\iota_1}, \ldots, \hat{p}_{\iota_{N_0}}$ satisfy

$$\frac{1}{N_0} \sum_{k=1}^{N_0} I\{\frac{M_0}{M} \hat{p}_{\iota_k} < x\} < x \tag{1}$$

for all $x \in [0, 1]$, where M_0 is the number of null SNPs. Given (1) holds, application of the original BH procedure[2] will control the false discovery rate.

In order to obtain a sample of the null SNPs, we have to rely on an independent set of screening p-values,

$$p_{i_1}^{val}, \ldots, p_{i_{N_v}}^{val}$$

obtained from the genotypes in the validation sample. Our goal is use these p-values to correctly select null SNPs from the validation SNPs. Inclusion of non-null SNPs in the set $\mathcal{S}_{prenull}$ of presumed nulls will drastically reduce power. Thus we use the BH procedure with a high q to select presumed null SNPs $s_{J_1}, \ldots, s_{J_A}$. A subset of $s_{J_1}, \ldots, s_{J_A}$ are true nulls: $s_{\eta_1}, \ldots, s_{\eta_B}$, with $\tilde{p}_{\eta_1} \leq \cdots \leq \tilde{p}_{\eta_B}$. If τ is small, then the joint distribution of $\eta_1, \ldots, \eta_B$ conditional on B is close to hypergeometric sampling from the set of nulls I_0.

Now condition on B, the number of null SNPs in the presumed nulls, and the values of the imputed p-values $\tilde{p}_{\iota_1}, \ldots, \tilde{p}_{\iota_{M_0}}$. Given $\eta_1, \ldots, \eta_B$, define the quantities

$$D_i = \sum_{j=1}^{M_0} I(\tilde{p}_{\iota_j} \leq \tilde{p}_{\eta_i})$$

These D_i are the ranks of the $\tilde{p}_{\eta_1}, \ldots, \tilde{p}_{\eta_B}$ within the null SNPs.

Under the hypergeometric sampling assumption, we provide upper bounds t_i for $i = 1, \ldots, A$ so that

$$\Pr[\frac{D_i}{M_0} > t_i \text{ for some } 1 \leq i \leq b | B = A] < \alpha_1$$

where α_1 is an error control parameter. *[Author's note: Work in progress.]*

For each SNP in $\mathcal{S}_{imputed}$, also define

$$R_i = \sum_{j=1}^{B} I(\tilde{p}_i \leq \tilde{p}_{\eta_j})$$

While R_i is unknown, we can compute an upper bound

$$\hat{R}_i = \sum_{k=1}^{A} I(\tilde{p}_i \leq \tilde{p}_{J_k})$$

The following observation motivates our procedure. Although $\eta_1, \ldots, \eta_B$ are unknown, at the very least, we know that $R_i \leq \hat{R}_i$. If we knew the values of D_i, then we could transform all of the imputed p-values by

$$\hat{p}_i' = \frac{D_{R_i}}{M_0}$$

and then we would have the property

$$\frac{1}{M_0} \sum_{i=1}^{M_0} I(\hat{p}_{\iota_i}' \leq x) \leq x$$

for all $x \in [0, 1]$, which means that type I error could be controlled by using the transformed p-values $\hat{p}_i'$. However, we only have approximate upper bounds d_i for the unknown quantities D_i/M_0 and R_i for $\hat{R}_i$. The transformed p-values thus take the form

$$\hat{p}_i = d_{R_i}$$

which satisfy the property (1), conditional on the event E that the upper bounds d_i hold: i.e., $D_i < d_i$ for all $i = 1, \ldots, B$.

Applying the BH(q) algorithm to the transformed p-values with threshold α_2 yields a false discovery rate of

$$\begin{aligned} FDR &= \mathbf{E}\left[\frac{V}{R}\right] \\ &= \mathbf{E}\left[\frac{V}{R}|E\right]\mathbf{Pr}[E] + \mathbf{E}\left[\frac{V}{R}|E^c\right]\mathbf{Pr}[E^c] \\ &\leq \mathbf{E}\left[\frac{V}{R}|E\right] + \mathbf{Pr}[E^c] \\ &\leq \alpha_2 + \alpha_1 \end{aligned}$$

5 Simulations

5.1 Synthetic data

Any serious algorithm for imputing genotypes must be quite complex, since it has to estimate the haplotype block structure of the population. Due to this, running and analyzing the imputation algorithm becomes one of the main technical challenges of running simulation studies of genotype imputation.

However, if we use a model in which the haplotype block structure is already known, and in which independence between blocks is enforced, the optimal imputation rule ends up being simple. This allows us to easily test various methodologies for drawing inferences from imputed data, at the expense of using a somewhat unrealistic model. Nevertheless, we can explore the relative impacts of the two of the problems listed in section 3: miscalled SNPs and inaccurate imputation (due to having a small reference panel sample), in addition to exploring the effects of sample size and number of tag SNPs used.

5.1.1 Population Generation

We consider a population of haploid individuals: the genotype X of each individual consists of K_b blocks of length L_b; i.e., each haplotype has a total of $M = K_b * L_b$ binary bases. Let H^j be the haplotype of the individual in the jth block. An individual is sampled by independently drawing a haplotype $H^1, \ldots, H^{K_b}$from each block. In fact, there exist N_{pop} possibly distinct haplotypes for each block, $H^j_1, \ldots, H^j_{N_{pop}}$, and the haplotype H^j for an individual for the jth block is drawn uniformly from those N_{pop} haplotypes. Let $\pi_R(g)$ be the probability mass function for a genotype g generated in this manner.

The distribution of haplotypes per block is generated by duplication-mutation process applied to a fixed population over several generations. In the mth generation, the jth haplotype block contains N_{pop} haplotypes $H^{j,m}_1, \ldots, H^{j,m}_{N_{pop}}$. Intially, all haplotypes are identically zero. However, at each iteration,

1. Each allele in each haplotype is mutated (toggled) with probability p_{mut}
2. $H^{j,m+1}$ is chosen from $H^{j,m}_1, \ldots, H^{j,m}_{N_{pop}}$ uniformly at random. This allows mutated haplotypes to be duplicated.

This process repeats for N_{gen} generations.

To generate the phenotype distribution, we randomly create a coefficient vector β and a constant β_0.

1. Let M_{cause} be the number of causative variants.
2. Intialize β to be a vector of length M consisting of all zeros. Randomly select a subset of the indices of β of size M_{cause}. Populate those entries with iid standard normal variates.
3. Manually choose a value for β_0: this controls how commonly the phenotype appears.

The vector β and constant β_0 define the distributions π^0_1 and π^0_0 by the following

$$\pi^0_1(g) \propto \pi_R(g) \frac{e^{\beta^T g + \beta_0}}{1 + e^{\beta^T g + \beta_0}}$$

$$\pi^0_0(g) \propto \pi_R(g) \frac{e^{-\beta^T g + -\beta_0}}{1 + e^{-\beta^T g + -\beta_0}}$$

Due to the computational difficulty of computing log-odds ratios from π^0_1 and π^0_0, we generate the actual case and control distributions π_1 and π_0 as follows:

- Sample a large number $N_{case,control}$ of individuals from π_R. Define π'_R as the uniform distribution over these individuals.
- Define π_0 by
$$\pi_1^0(g) \propto \pi'_R(g) \frac{e^{\beta^T g + \beta_0}}{1 + e^{\beta^T g + \beta_0}}$$
- Define π_1 by
$$\pi_1^0(g) \propto \pi'_R(g) \frac{e^{\beta^T g + \beta_0}}{1 + e^{\beta^T g + \beta_0}}$$

This allows us to compute the true log-odds ratios exactly.

To simulated the effect of miscalled SNPs, we define modified distributions $\pi_{0,x}, \pi_{0,x}$. To sample g from $\pi_{i,x}$, draw g' from π_i, but then randomly toggle each allele in g' with probability x to obtain g.

5.1.2 Simulated Experiments

A reference panel is given consisting of either the complete set of haplotypes for each block (*perfect imputation*), or a subsample of N_{ref} haplotypes for each block. Let $\eta_1, \ldots, \eta_{N_{ref}}$ denote the indices of the subsampled haplotypes: hence, the reference panel consists of $X^j_{\eta_1}, \ldots, X^j_{\eta_{N_{ref}}}$ for each block.

Tag SNPs are selected based on the reference panel. For each block, we select K_{tag} tag SNPs. The selection is done via a greedy algorithm.

Given a sample size L and a miscall probability p_{mis}, we generate complete genotypes $X_1, \ldots, X_L$ cases iid from $\pi_{1,p_{mis}}$ and $X_{L+1}, \ldots, X_{2L}$ controls iid from $\pi_{0,p_{mis}}$. This yields complete p-values $p_1, \ldots, p_M$ for each SNP. Meanwhile, using the tag SNPs, imputed genotypes $\tilde{X}_1, \ldots, \tilde{X}_{2L}$ are obtained. The imputation rule is as follows.

Imputation Rule

- Iterate for each block $j = 1, \ldots, K_b$:
- Let $t_1, \ldots, t_{K_{tag}}$ be the tag SNPs for the jth block. These tag SNPs partition the reference into D classes, $C_1, \ldots, C_D$, in the sense that h^j_k, h^j_l are in the same class if and only if h^j_k matches h^j_L exactly on the set of tage SNPs. Collect class-specific means $m_1, \ldots, m_D$ by averaging,
$$\mu_o = \frac{1}{|C_o|} \sum_{h \in C_o} h$$

Also define the global mean,

$$\mu = \frac{1}{N_{pop}} \sum_{k=1}^{N_{pop}} h_k^j$$

- Determine the class that the ith genotype X_i falls in. If the X_i falls in class C_o, impute the jth block of X_i by μ. If X_i does not fall in any of the classes $C_1, \dots, C_D$, impute the non-tag SNP entries of the jth block of X_i by μ.

From imputed genotypes $\tilde{X}_1, \dots, \tilde{X}_{2L}$, obtain imputed p-values $\tilde{p}_1, \dots, \tilde{p}_M$.

We apply the original BH procedure to the imputed p-values and assess the true false discovery proportion. Also, for different values of L_{val} and N_v, we apply our proposed testing procedure for imputed data (Section 3).

5.1.3 Results

Initial parameters

We generate the population of haplotypes with $K_b = 100$, $L_b = 10$, $N_{pop} = 50$, $N_{gen} = 30$, $p_{mut} = .1$. The coefficient vector for the phenotype is generated with $M_{cause} = 2$ nonzero entries, both drawn from $N(0, \sqrt{10})$, and β_0 is chosen so that the proportion of cases in the reference population is 0.2. The case and control populations are generated with $N_{large} = 1000$. In our code, `impute.R`, we set the random seed to 1 for purposes of fixing the reference, case and control. We do not control the random seed for the rest of the simulation.

Figure 4(i) displays the true absolute log odds ratios of the SNPs, when sorted. Setting $\tau = 0.1$ results in $M_1 = 141$ non-null SNPs. Figure 4 (ii) illustrate the relationship between imputation accuracy and the number of tag SNPs when the reference data is used to impute itself. Figure 4 (iii) displays the minor allele frequency of the reference population.

Using the full reference panel with no errors, and 4 tag SNPs per block, we have significant discordance between true log odds ratios and asymptotic imputed data log odds ratios (Figure 5(i)), with 63 systematically induced false positives. However, we will see that given a realistic sample size, the false positive rate may be much lower.

In one run, we use $L = 10000$ cases and the same number of controls, with 4 tag SNPs per block. Figures 5(ii) and (iii) show the false discovery

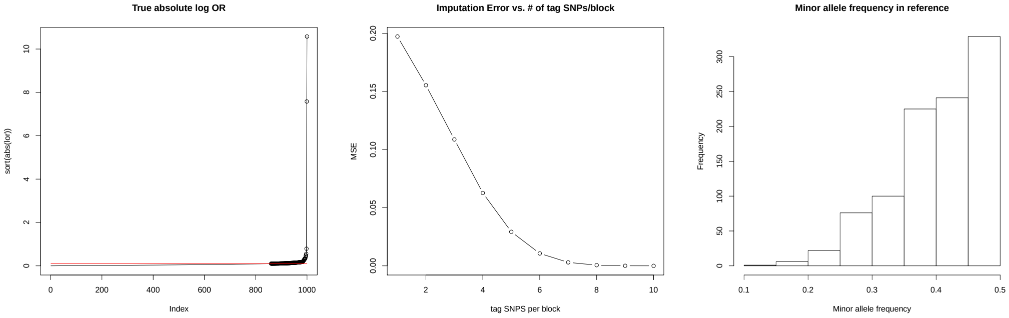


Figure 4: *Left* (i). True absolute log odds ratios of SNPs (sorted) *Center* (ii) Imputation error vs number of tag SNPs per haplotype Block. Error is measured by average squared error. *Right* (iii) Minor allele frequency in reference

proportion (fdp) curves for genotyped data and imputed data, respectively. Notable is the fact that the fdp curve for imputed data is rougher, a trend that becomes increasingly evident when the sample size increases.

We set $L_{val} = 1000$, and false discovery rate threshold to $q = 0.2$. We compare the following procedures

- BH(q) on the complete genotyped data
- BH(q) on the data imputed using the given tag SNPs
- Our method on the imputed data

The following table shows the results of a few repeated runs. Recall R is the number of rejections, V the number of false rejections, and $fdp = V/R$.

Run	Full data R	V	fdp	Imputed R	V	fdp	Our method R	V	fdp
1	22	0	0	11	0	0	8	0	0
2	20	0	0	9	0	0	7	0	0
3	20	0	0	10	0	0	8	0	0

In summary, neither the naive method nor our method makes any false rejections, though our method has less power.

Increased sample size

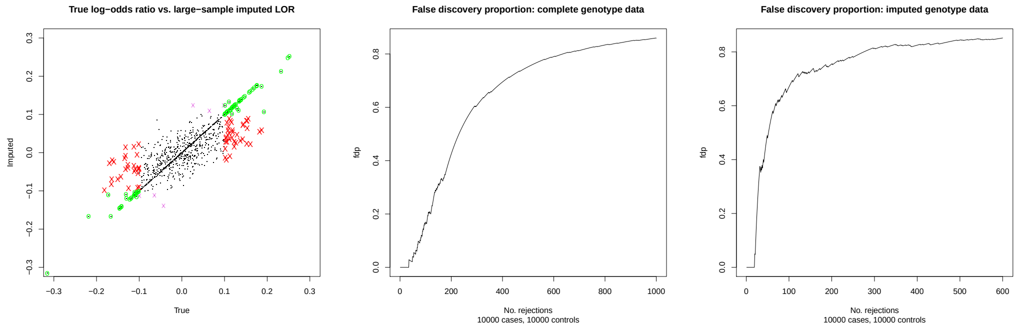


Figure 5: *Left* (i). True log odds ratios of SNPs vs. asymptotic imputed log odds ratios, 4 tag SNPS/block. Green circles=true positives, violet crosses=false negatives, red crosses=false positives. *Center* (ii) False discovery proportion of genotyped data, $L = 1000$ *Right* (iii) False discovery proportion of imputed data, $L = 1000$, 4 tag SNPS/block

Increasing the sample size a hundred fold to $L = 1000000$ and $L_{val} = 10000$, problems start to occur for the naive method. However, our method continues to be quite conservative.

Run	Full data R	V	fdp	Imputed R	V	fdp	Our method R	V	fdp
1	111	0	0	29	6	0.21	13	1	0.07
2	112	0	0	29	6	0.21	13	1	0.07
3	111	0	0	28	6	0.21	14	1	0.07

Notice that at this sample size, the sampling error has little influence compared to the systematic bias induced by the imputation procedure.

Incomplete reference panel

Return to $L = 10000$ and $L_{val} = 1000$, but this time construct the reference panel by a subsample of size 25. Systematically induced false positives increase to 75 while asymptotically detectible true positives decrease to 19. Power for both the naive method drops slightly, more for our method. Yet no false rejections are made.

Run	Full data R	V	fdp	Imputed R	V	fdp	Our method R	V	fdp
1	25	0	0	7	0	0	6	0	0
2	25	0	0	6	0	0	6	0	0
3	30	0	0	7	0	0	0	0	0

Incomplete reference, more tag SNPs

Here is a scenario in which the effects of Pattern I manifest. Return to $L = 10000$ and $L_{val} = 1000$, and As in the last section, our reference panel is a subsample of size 25 of the true reference panel, but now increase the number of tag SNPs to 6 per block.

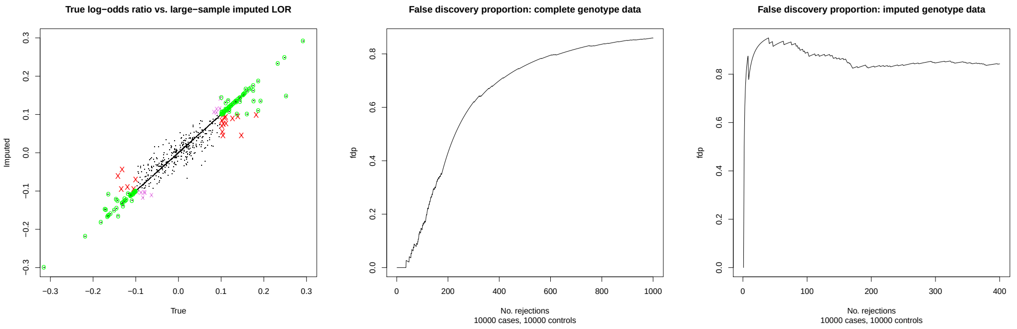


Figure 6: *Left* (i). True log odds ratios of SNPs vs. asymptotic imputed log odds ratios, 6 tag SNPS/block. Green circles=true positives, violet crosses=false negatives, red crosses=false positives. *Center* (ii) False discovery proportion of genotyped data, $L = 1000$ *Right* (iii) False discovery proportion of imputed data, $L = 1000$, 6 tag SNPS/block

The large-sample number of false positives due to imputation errors reduces to 19, and the number of true positives detectible in the imputation data increases to 44. Yet surprisingly, the false positive rates rise for the naive method.

Run	Full data R	V	fdp	Imputed R	V	fdp	Our method R	V	fdp
1	21	0	0	7	6	0.85	0	0	0
2	30	0	0	11	9	0.81	0	0	0
3	25	0	0	9	8	0.88	0	0	0

This phenomenon can be explained, however. We have removed additional tag SNPs from the list of SNPs to be be tested and thus depleted the data of many strong signals. On the other hand, having more tag SNPs, up to a certain point, increases the prospect of Pattern I (§3.1) occurring, since Pattern I depends on the existence of two haplotypes which are indistinguishable given the typed SNPs, but which are distinguished from the other haplotypes in the population. Figure 6 provides a comparison with with Figure 5.

While the naive method is plagued with almost as many false discoveries as true ones, our method controls the type I error by making no rejections.

6 Discussion

Our simulations show that in certain conditions, the naive method for testing imputed data can have some degree of robustness against incomplete reference data. However, rather counterintuitively, a large sample size, and a relatively large number of tag SNPs can lead to dramatically increased type I error rates for naive testing of associations based on imputed data.

Given the difficulty of gauging one's vulnerability to increased type I error from using imputed data, it would be prudent to obtain some form of additional validation data to check for the prevalence of systematic error induced by imputation. Our method is a formal way to do this– it has some degree of guaranteed type I error control, but it requires huge sample sizes to be truly effective, and is often overconservative to the extreme in common situations.

It remains for future work to develop a method which can control type I error for imputed data, and yet remain competitive against the naive methods in situations where excessive caution is unwarranted.

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