Reducing bias of allele frequency estimates by modeling SNP genotype data with informative missingness

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INTRODUCTION

Even with the advancement of biological technologies, genotype missingness is still common in practice. Genotype missingness can be caused by damage or loss in performance of probes in multiplexed genotyping platforms, or variation in DNA quality or molecular effects. In fact, the presence of missing genotypes is unavoidable in even carefully designed studies such as the HapMap project (HapMap, 2005) and the genome-wide association study (GWAS) by the Wellcome Trust Case Control Consortium (2007).

For studies with high-density single-nucleotide polymorphisms (SNPs), a popular approach to deal with genotype missingness is to impute missing genotypes before performing analyses (Stephens and Scheet, 2005; Marchini et al., 2007; Yu and Schaid, 2008). However, for studies with low-density SNPs, the most commonly used approach to dealing with genotype missingness is to simply remove the observations with missing genotypes from the analyses. This naïve method is straightforward but is valid only when the missingness is random. However, a given assay often has a different capability in genotyping heterozygotes and homozygotes, causing the phenomenon of “differential dropout” in the sense that the missing rates of heterozygotes and homozygotes are different. In practice, differential dropout among genotypes exists in even carefully designed studies, such as the data from the HapMap project and the Wellcome Trust Case Control Consortium. Under the assumption of Hardy–Weinberg equilibrium and no genotyping error, we here propose a statistical method to model the differential dropout among different genotypes. Compared with the naïve method, our method provides more accurate allele frequency estimates when the differential dropout is present. To demonstrate its practical use, we further apply our method to the HapMap data and a scleroderma data set.

Keywords: allele frequency, EM algorithm, genotype, informative missingness, missing at random, single-nucleotide polymorphism

The presence of missing single-nucleotide polymorphism (SNP) genotypes is common in genetic studies. For studies with low-density SNPs, the most commonly used approach to dealing with genotype missingness is to simply remove the observations with missing genotypes from the analyses. This naïve method is straightforward but is valid only when the missingness is random. However, a given assay often has a different capability in genotyping heterozygotes and homozygotes, causing the phenomenon of “differential dropout” in the sense that the missing rates of heterozygotes and homozygotes are different. In practice, differential dropout among genotypes exists in even carefully designed studies, such as the data from the HapMap project and the Wellcome Trust Case Control Consortium. Under the assumption of Hardy–Weinberg equilibrium and no genotyping error, we here propose a statistical method to model the differential dropout among different genotypes. Compared with the naïve method, our method provides more accurate allele frequency estimates when the differential dropout is present. To demonstrate its practical use, we further apply our method to the HapMap data and a scleroderma data set.

Keywords: allele frequency, EM algorithm, genotype, informative missingness, missing at random, single-nucleotide polymorphism

The majority of imputation methods utilize the information of linkage disequilibrium (LD) between SNPs to reconstruct haplotypes and to infer missing genotypes (Stephens and Scheet, 2005; Marchini et al., 2007; Yu and Schaid, 2007; Zhao et al., 2008; Calus et al., 2011; Daetwyler et al., 2011). The major limitation of these methods is that they are not aware of the differential dropout phenomenon. This phenomenon is called “differential dropout,” which exists in some genotyping platforms, or variation in DNA quality or molecular effects. In fact, the presence of missing genotypes is unavoidable in even carefully designed studies such as the HapMap project (HapMap, 2005) and the genome-wide association study (GWAS) by the Wellcome Trust Case Control Consortium (2007).
real data sets such as the HapMap (2005) and the WTCCC data (Wellcome Trust Case Control Consortium, 2007). Hao and Cawley (2007) evaluated the impacts of differential dropout among SNP genotypes on association tests, and they found that differential dropout can cause undesirable outcomes to association tests. In this work, we show that differential dropout can bias the estimates of allele frequencies even when the missingness is informative, and it can simultaneously estimate the missing rates of homozygotes and heterozygotes. In addition, we apply our method to the HapMap data and a scleroderma data set to demonstrate its utility.

**MATERIALS AND METHODS**

**MISSING DATA MODEL**

Given a SNP with two alleles, A and B, there are three possible genotypes (AA, BB, and AB). Studies have shown that homozygotes and heterozygotes often have different dropout rates due to various reasons such as today’s automated genotyping technologies (Oliphant et al., 2002; Allen et al., 2003; Kang et al., 2004; Matsuzaki et al., 2004). Under the assumption of no genotyping error, Table 1 presents the probabilities of observing the three genotypes and the missingness, given the true genotypes. We define the missing rates of homozygotes and heterozygotes as follows:

\[ \alpha_{\text{Hom}} = \Pr (O = ?? | T = AA \text{ or } BB) , \]

\[ \alpha_{\text{Het}} = \Pr (O = ?? | T = AB) , \]

where “??” represents missingness in genotype, \( \alpha_{\text{Hom}} \) is the missing rate of homozygotes (AA or BB), \( \alpha_{\text{Het}} \) is the missing rate of heterozygotes (AB), T means True genotype, and O means Observed genotype. Note that the assumption of MAR holds when \( \alpha_{\text{Hom}} = \alpha_{\text{Het}} \) (no differential dropout). However, differential dropout is a common phenomenon in real data (Hao and Cawley, 2007), so we allow \( \alpha_{\text{Hom}} \neq \alpha_{\text{Het}} \) in our model.

**ESTIMATION OF ALLELE FREQUENCIES**

Given a SNP with two alleles, A and B, there are four possible outcomes when observing the genotypes (AA, ??, AB, and BB). As shown by Table 1, the observed counts of the four categories are \( n_1, n_2, n_3, \) and \( n_4 \), respectively. A constraint among these four observed counts is \( n_1 + n_2 + n_3 + n_4 = n \), where \( n \) is the total number of subjects. The three unknown parameters that need to be estimated are \( \alpha_{\text{Hom}}, \alpha_{\text{Het}}, \) and \( p_A \), where \( p_A \) is the frequency of allele A. Under the assumption of no genotyping error and the Hardy–Weinberg equilibrium (HWE) for the true genotype distribution, the probability of observing genotype AA is

\[ p_1 = \Pr (O = AA | T = AA) = \Pr (O = AA | T = AA) \cdot \Pr (T = AA) \]

\[ = (1 - \alpha_{\text{Hom}}) \cdot p_A^2 \]

Similarly, we have

\[ p_2 = \Pr (O = ?? | T = AA) = \alpha_{\text{Hom}} p_A^2 + \alpha_{\text{Hom}} (1 - p_A)^2 \]

\[ + \alpha_{\text{Het}} 2p_A (1 - p_A) , \]

\[ p_3 = \Pr (O = AB | T = AA) = (1 - \alpha_{\text{Het}}) 2p_A (1 - p_A) , \]

\[ p_4 = \Pr (O = BB | T = AA) = (1 - \alpha_{\text{Hom}})(1 - p_A)^2 . \]

The observed-data likelihood is

\[ L_{\text{OBS}} \propto p_1^{n_1} \cdot p_2^{n_2} \cdot p_3^{n_3} \cdot p_4^{n_4} , \]

and the log-likelihood is

\[ \log L_{\text{OBS}} \propto n_1 \log (1 - \alpha_{\text{Hom}}) + 2 \log p_A \]

\[ + n_2 \log \left[ \alpha_{\text{Hom}} p_A^2 + \alpha_{\text{Hom}} (1 - p_A)^2 + \alpha_{\text{Het}} 2p_A (1 - p_A) \right] \]

\[ + n_3 \log (1 - \alpha_{\text{Het}}) + 2 \log p_A + \log (1 - p_A) \]

\[ + n_4 \log (1 - \alpha_{\text{Hom}}) + 2 \log (1 - p_A) \] .

Given the observed genotype distribution \( (n_1, n_2, n_3, \) and \( n_4) \) and a constraint \( (n_1 + n_2 + n_3 + n_4 = n) \), the three parameters \( (\alpha_{\text{Hom}}, \alpha_{\text{Het}}, \) and \( p_A) \) are identifiable and can be estimated with the expectation–maximization (EM) algorithm (Dempster et al., 1977). In the Appendix, we show that these three parameters are identifiable. Note that this method is developed under the assumption of HWE and no genotyping error. With genotyping errors, the model will become much more complicated (Liu et al., 2009b). More parameters will be involved in the model and these parameters \( (> 3) \) will no longer be identifiable.

**RESULTS**

**SIMULATION STUDY: ESTIMATION OF ALLELE FREQUENCIES**

Following Hao and Cawley (2007), we define a differential dropout ratio (DDR) as

\[ r_{\text{drop}} = \frac{\alpha_{\text{Het}}}{\alpha_{\text{Hom}}} , \]

where \( \alpha_{\text{Het}} \) and \( \alpha_{\text{Hom}} \) are the missing rates of heterozygotes and homozygotes, respectively. We simulated a SNP with a minor allele frequency (MAF) of 0.1 and assumed HWE at this SNP. The overall genotype missing rates were set at 0.02, 0.05, 0.1, and 0.15, respectively. The DDRs were specified at 0.25, 0.5, 1, 2.5, 5, and 10, respectively. The total sample size was set at 2,000. We compared our method with the naïve method that simply removed the observations with missing genotypes from the analyses. With

| Observed/True | AA | ?? | AB | BB |
|---------------|----|----|----|----|
| AA            | 1 - \( \alpha_{\text{Hom}} \) | \( \alpha_{\text{Hom}} \) | 0 | 0 |
| BB            | 0 | \( \alpha_{\text{Hom}} \) | 0 | 1 - \( \alpha_{\text{Hom}} \) |
| AB            | 0 | \( \alpha_{\text{Het}} \) | 1 - \( \alpha_{\text{Het}} \) | 0 |
| Probability   | \( p_1 \) | \( p_2 \) | \( p_3 \) | \( p_4 \) |
| Count         | \( n_1 \) | \( n_2 \) | \( n_3 \) | \( n_4 \) |

The first row lists the four possible outcomes of the observed genotypes, including the three genotypes and the missingness (??). The first column lists the three true genotypes. Each element in this table is the probability of observing some genotype given the true genotype.
1,000 replications, Figure 1 presents the box-and-whiskers plots of the 1,000 estimates of allele frequencies. We can see that when DDR = 1 ($\alpha_{\text{Hom}} = \alpha_{\text{Het}}$, no differential dropout), both the naïve method and our new method give unbiased estimates of allele frequencies (in our simulation results, the medians and means are very close). When DDR < 1 or > 1, the naïve method gives biased estimates while the new method still generates unbiased results. The more the DDR departs from 1, the more biased are the estimates that the naïve method produces. This bias is especially prominent when the overall genotype missing rate is equal to or larger than 0.05. We also simulated a SNP with MAF of 0.2, and the result was very similar to that shown in Figure 1 (of course the centers of the boxes changed to 0.2).

Although our method can improve the accuracy, the precision (inverse variance) of the allele frequency estimates is lowered. Therefore, when DDR = 1, the naïve method is superior to our method, with consideration of both the accuracy and the precision. This is expected because our method involves more parameters ($\alpha_{\text{Hom}}$, $\alpha_{\text{Het}}$, and $p_A$) than does the naïve method ($p_A$), which lowers the precision of estimates in our method.

**SIMULATION STUDY: IMPACT OF HARDY–WEINBERG DISEQUILIBRIUM**

To evaluate the sensitivity of our method to the assumption of HWE, we performed a simulation study to examine the bias of allele frequency estimates when the assumption of HWE does not hold. The probabilities of the true genotype being AA, AB, and BB can be represented as:

- $\Pr(T = AA) = p_A^2 + p_A (1 - p_A) f$,
- $\Pr(T = AB) = 2p_A (1 - p_A) (1 - f)$,
- $\Pr(T = BB) = (1 - p_A)^2 + p_A (1 - p_A) f$,

where $f$ is the fixation index (Weir, 1996; Wakefield, 2010), a measure of the departure from HWE. When $f = 0$, there is no departure from HWE. The larger the departure of $f$ from 0, the larger the degree of HWD. When $f$ is positive, the departure from HWE results in excess homozygosity. When $f$ is negative, the departure from HWE results in excess heterozygosity. We simulated a SNP with MAF of 0.1. The total sample size was set at 2,000. Following the setting of fixation index when Chen and Kao (2006) examined the sensitivity of their method to the assumption of HWE, we also...
evaluated the performance of our method with the fixation index \( f \) of 0.1 and 0.2. Figures 2 and 3 present the box-and-whiskers plots of the 1,000 estimates of allele frequencies when the fixation index \( f = 0.1 \) and 0.2, respectively. We can see that our method leads to an upward bias to the allele frequency estimates when \( f > 0 \), and a downward bias when \( f < 0 \) (result not shown). Our method is not very robust to the assumption of HWE. This is a caution when applying this approach.

For each scenario in Figures 2 and 3, we also provided the mean of the 1,000 \( P \) values of the exact test for HWE for the 1,000 replications. Using the observed counts of genotypes AA, AB, and BB, the exact test for HWE (Wigginton et al., 2005) was performed with the R package “Hardy–Weinberg” (Grafen and Camarena, 2008). When the fixation index \( f = 0.2 \) (Figure 3), although our method leads to a large upward bias to the allele frequency estimates, the \( P \) values of the exact test for HWE are extremely small (\(< 1.3 \times 10^{-4}\)). Investigators provided with such significant results for the HWE tests should avoid using our approach. Given the fixation index \( f = 0.1 \) (Figure 2), the HWE tests may fail to give researchers an alarm when the overall genotype missing rate (P.drop) is large (0.15) and the DDR is small (0.25). This is because a small DDR (<1) indicates a larger missing rate for homozygotes than for heterozygotes, which dilutes the excess homozygosity linking to a positive fixation index \( f \). If, unfortunately, the overall genotype missing rate (P.drop) is large (here, 0.15), the dilution of excess homozygosity will be even more pronounced. This inconsistency between the observed genotype distribution and the true genotype distribution will induce a blind spot of the HWE test. Nonetheless, overall, performing a HWE test on the observed genotype distribution is a way to evaluate the appropriateness of using our approach.

**APPLICATION TO HAPMAP DATA**

We further applied our method to the HapMap data (HapMap, 2005). We downloaded the chromosome 17 genotype data of the 45 Chinese and 44 Japanese released in October, 2005 (HapMap, 2005). We estimated the missing rates of homozygotes (\( \alpha_{\text{Hom}} \)) and the missing rates of heterozygotes (\( \alpha_{\text{Het}} \)) of these HapMap SNPs, by using our method. After removing SNPs without missing genotypes, we estimated the \( \alpha_{\text{Hom}} \)'s and \( \alpha_{\text{Het}} \)'s of the remaining SNPs. Figure 4 presents the histograms of the \( \hat{\alpha}_{\text{Hom}} \) and \( \hat{\alpha}_{\text{Het}} \) for the Chinese and Japanese samples, respectively. We can see that the
missing rates of heterozygotes are generally larger than the missing rates of homozygotes (DDR > 1), for both the Chinese and Japanese samples. Hao and Cawley (2007) used the Affymetrix genotypes that present no evidence of DDR as benchmark to obtain an estimate of \( r_{\text{drop}} \) as 1.73 in the HapMap data. With our method, we estimate \( r_{\text{drop}} \) as 1.81 and 1.97 for the Chinese and Japanese samples, respectively.

Figure 5 shows the interval of point estimate ± standard error of missing rate for each HapMap SNP on chromosome 17. If we approximate the confidence intervals of missing rates with point estimates ± 2 × standard errors, all SNPs have overlapped confidence intervals of missing rates for homozygotes and for heterozygotes. For these SNPs, the missing rates for homozygotes and for heterozygotes are not significantly different. This is not unexpected given the small sample sizes of the HapMap data (∼45).

APPLICATION TO SCLERODERMA DATA

Scleroderma is a chronic autoimmune disease characterized by skin thickening and vascular abnormalities. Scleroderma has an estimated prevalence of 250 patients per million in the United States (Adnan, 2008). There are two main subtypes of scleroderma: diffuse scleroderma and limited scleroderma (Nikpour et al., 2010). The studied data set contains genetic and clinical information collected from the Scleroderma Family Registry and DNA Repository at the University of Texas Health Science Center at Houston (Baugh et al., 2002; Wu et al., 2006). The majority of subjects are white Caucasians. Therefore, we only include the 655 white Caucasians into the analysis. Among the 655 subjects, 160 were diagnosed with diffuse scleroderma, 266 subjects with limited scleroderma, and the remaining 229 subjects were healthy controls. A G/C SNP at position −173 (rs755622) in the 5′ promoter region of migration-inhibitory factor (MIF) was genotyped for each of the 655 subjects. Among the 655 subjects, 15 subjects were missing at SNP rs755622 (MIF −173 SNP), including 1 (0.63%) patient with diffuse scleroderma, 12 (4.51%) patients with limited scleroderma, and 2 (0.87%) controls. We selected the limited scleroderma patient group (to be the case group) and the control group for the two-sample analysis.

First, we applied the naïve method to the data, by simply removing the subjects with missing genotypes from the analysis. The MAFs in the case and control groups are 12.6 and 18.5%, respectively.

FIGURE 3 | The box-and-whiskers plots of 1,000 estimates of MAF, given MAF = 0.1 and the fixation index \( f = 0.2 \). The different panels in the figure are arranged so that the overall genotype missing rate (\( P_{\text{drop}} \)) is 0.02, 0.05, 0.1, and 0.15 (from top to bottom) and the DDR (\( r_{\text{drop}} \)) is 0.25, 0.5, 1, 2.5, 5, and 10 (from left to right). Below each panel, we list the mean of the 1,000 \( P \) values of the exact test for Hardy–Weinberg equilibrium for the 1,000 replications.
FIGURE 4 | Histograms of missing rates for homozygotes and heterozygotes in the HapMap Chinese and Japanese samples.

respectively. The \( P \) value of Fisher’s exact test for allelic association is 0.012. Then we applied our method to the data. Based on the observed genotype distribution (AA, AB, and BB), the MAFs in the case and control groups are estimated as 12.1 and 18.4%, respectively. The Fisher’s exact test for allelic association yields a \( P \) value of 0.007. Given a significance level of 0.05, both the naïve method and our method suggest an association of the MIF \(-173\) SNP with scleroderma. Our method gives very similar results with the naïve method. This is expected because the missing rates of genotypes are not high in this example. However, our method provides stronger evidence for the association of the MIF \(-173\) SNP with scleroderma that was recently confirmed by a study based on a large European population (Bossini-Castillo et al., 2011). The role of the MIF \(-173\) SNP in scleroderma deserves further investigation.

DISCUSSION

The presence of missing genotypes is common in genetic data. For studies with low-density SNPs, the most commonly used approach for dealing with genotype missingness is to simply remove the observations with missing genotypes from the analyses (Wu et al., 2006). This naïve method is valid only when there is no differential dropout among genotypes. However, in practice, a given assay often has a different capability in typing heterozygotes and homozygotes. Differential dropout among genotypes (a type of informative missingness) is detectable even in data from some carefully designed studies such as the HapMap (HapMap, 2005; Hao and Cawley, 2007) and the WTCCC projects (Wellcome Trust Case Control Consortium, 2007). Although the issue of informatively missing genotypes has been investigated in case-parent study design (Allen et al., 2003; Chen, 2004) and in haplotype data analyses (Liu et al., 2006, 2009a), there is no investigation of this issue on one locus for samples of unrelated individuals.

In this work, we propose a statistical method to estimate allele frequencies and missing rates of heterozygotes and homozygotes, under the assumption of HWE. We perform simulations to compare our method with the naïve method in the estimation of allele frequencies. Under HWE, our method gives accurate estimates for MAFs, with or without differential dropout among genotypes. The naïve method generates accurate estimates for MAFs only when there is no differential dropout among genotypes. We further apply our method to the HapMap data, and obtain similar estimates of DDRs to that estimated by Hao and Cawley (2007) with extra data. In addition, we analyze a scleroderma data set (Baugh et al., 2002; Wu et al., 2006) to show the practical use of our method. In contrast to Hao and Cawley (2007), we do not need to genotype
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FIGURE 5 | Missing rates for homozygotes and heterozygotes in the HapMap Chinese and Japanese samples. Each interval presents the point estimate ± standard error of missing rate for homozygotes or for heterozygotes of a SNP.

the sample with another gene chip and assume it as the ground truth in order to estimate the DDR. To the best of our knowledge, our method is the only statistical method to handle differential dropout among genotypes on one locus for samples of unrelated individuals.

Despite these merits, there are some limitations in our method. First, although the estimates of MAFs are unbiased under HWE, the precision of the estimates is lowered because our method involves more parameters in the estimation process. Therefore, when there is no differential dropout among genotypes (DDR = 1), the naive method may perform better than our method, considering both the accuracy and precision of the estimates. Second, our method relies on the assumption of HWE in the true genotype distribution. Although the true genotype distribution is unknown, one way to evaluate the appropriateness of using our approach is to perform a HWE test on the observed genotype distribution.

In this work, we focus on the estimation of allele frequencies, which is an important issue in epidemiological studies (Taioli et al., 2004). A future direction would be to evaluate the performance of our method and the naive method on association testing.

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APPENDIX
The identifiability of the three model parameters ($\alpha_{\text{Hom}}$, $\alpha_{\text{Het}}$, and $p_A$)

For the model shown in Table 1, we assume that none of $p_1$, $p_2$, $p_3$, and $p_4$ are zero. We have

\[ p_1 = \Pr(O = AA) = (1 - \alpha_{\text{Hom}}) \cdot p_A^2 \]  \hspace{1cm} (A1)
\[ p_2 = \Pr(O = AB) = \alpha_{\text{Hom}} p_A^2 + \alpha_{\text{Hom}} (1 - p_A)^2 + \alpha_{\text{Het}} 2p_A (1 - p_A) \]  \hspace{1cm} (A2)
\[ p_3 = \Pr(O = AB) = (1 - \alpha_{\text{Het}}) 2p_A (1 - p_A) \]  \hspace{1cm} (A3)
\[ p_4 = \Pr(O = BB) = (1 - \alpha_{\text{Hom}}) (1 - p_A)^2 \]  \hspace{1cm} (A4)

Define $C = \sqrt{\frac{p_3}{p_1}} = \frac{p_A}{\sqrt{p_1 p_4}}$. Then we have $p_A = \frac{C}{1 + C} = \frac{\sqrt{p_1}}{\sqrt{p_1} + \sqrt{p_4}}$.

From (A3), we have

\[ \alpha_{\text{Het}} = 1 - \frac{p_3}{2p_A (1 - p_A)} = 1 - \frac{p_3}{2 \left( \frac{C}{1 + C} \right) \left( \frac{1}{1 + C} \right)} \]
\[ = 1 - \frac{p_3 (\sqrt{p_1} + \sqrt{p_4})^2}{2 \sqrt{p_1 p_4}}. \]

Similarly, from (A1), we have

\[ \alpha_{\text{Hom}} = 1 - \frac{p_1}{p_A^2} = 1 - \frac{p_1}{C^2 (1 + C)^2} = 1 - \frac{p_1 (1 + C)^2}{C^2} \]
\[ = 1 - p_4 (1 + C)^2 = 1 - (\sqrt{p_1} + \sqrt{p_4})^2. \]

The three parameters ($\alpha_{\text{Hom}}$, $\alpha_{\text{Het}}$, and $p_A$) are thus all identifiable.