Imputation of coding variants in African Americans: better performance using data from the exome sequencing project

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ABSTRACT
Summary: Although the 1000 Genomes haplotypes are the most commonly used reference panel for imputation, medical sequencing projects are generating large alternate sets of sequenced samples. Imputation in African Americans using 3384 haplotypes from the Exome Sequencing Project, compared with 2184 haplotypes from 1000 Genomes Project, increased effective sample size by 8.3–11.4% for coding variants with minor allele frequency <1%. No loss of imputation quality was observed using a panel built from phenotypic extremes. We recommend using haplotypes from Exome Sequencing Project alone or concatenation of the two panels over quality score-based post-imputation selection or IMPUTE2’s two-panel combination.

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1 INTRODUCTION

Genotype imputation is a common practice for both genotyping (De Bakker et al., 2008; Li et al., 2009; Marchini and Howie, 2010) and sequencing studies (Fridley et al., 2010; Li et al., 2011). Increasingly large reference panels available in the public domain [e.g. those from the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2010, 2012) and UK10K project (Futema et al., 2012)] together with improved statistical methods (Howie
et al., 2012; Liu et al., 2013) have enhanced imputation quality, especially for rare variants with minor allele frequency (MAF) <5%. These improvements have resulted in both discovery and refined mapping of association with complex traits (Auer et al., 2012; Holm et al., 2011; Huang et al., 2012). However, few studies have examined the use of large study-specific reference panels, particularly the use of exome sequencing-derived panels in admixed populations. Here, we present a new resource for imputation in African Americans, built from 1692 African Americans sequenced by the Exome Sequencing Project (ESP) (Tennen et al., 2012). We assessed the use of the ESP data as an imputation reference panel and compared the results with those obtained using the 1000 Genomes Project Phase1 (1000G; version 3, March 2012 release) (The 1000 Genomes Project Consortium, 2012) data. Additionally, we evaluated the potential consequences of using a reference panel built from samples selected on the basis of phenotypic extremes or disease status instead of a population-based random sample. Lastly, we compared multiple approaches to combine the ESP and 1000G panels for the imputation of rare coding variants.

2 METHODS

2.1 Exome Sequencing Project

2.1.1 ESP and African American Participants The complete ESP dataset (Fu et al., 2012) consists of whole exome data for 6823 individuals. Samples were sequenced at the University of Washington (SeattleGO) and the Broad Institute (BroadGO). Among the 6823 individuals, 1692 participants were African Americans with genome-wide association data available for analysis. The 1692 African Americans ESP samples include 845 from the Women’s Health Initiative (WHI) study (The Women’s Health Initiative Study Group, 1998) as part of the WHI Sequencing Project (WHISP), and a total of 847 including Atherosclerosis Risk in Communities (ARIC; Muntaner study (The Women’s Health Initiative Study Group, 1998) as part of ESP samples include 845 from the Women’s Health Initiative (WHI) SNP Health Association Resource study. Before phasing and imputation, we removed Affymetrix 6.0 SNPs with genotype call rates <90%, or Hardy–Weinberg exact test (Wigginton et al., 2005) $P<10^{-6}$ or MAF <1%. QC details were described previously (Auer et al., 2012; Reiner et al., 2011).

2.1.2 ESP ‘Extreme’ and ‘Normal’ Panel Construction The 1692 ESP African Americans were selected based on the following phenotypic traits: (i) LDL (N=254; 131 with high LDL and 123 with low LDL), (ii) blood pressure (N=247: 132 with high blood pressure and 115 with low blood pressure), (iii) body mass index (BMI, N=609: 429 with high BMI and 180 with normal to low BMI), (iv) early onset MI (EOMI, N=324: 39 EOMI cases and 285 EOMI controls), (v) stroke (N=40, all cases) and (vi) random samples (N=218). We constructed one ESP ‘Extreme’ panel and one ESP ‘Normal’ panel each with 853 individuals. The ESP ‘Extreme’ panel included (i) 254 individuals with high/low LDL (131 with high LDL and 123 with low LDL), (ii) 247 individuals with high/low blood pressure (132 with high blood pressure and 115 with low blood pressure), (iii) 40 stroke cases, (iv) 39 EOMI cases and (v) 273 individuals with high BMI. The ESP ‘Normal’ panel consists of 80% individuals with ‘non-extreme’ phenotypes and 20% with extreme phenotypes so as to better represent a population sample. Individuals with ‘non-extreme’ phenotypes (N=683) are from random sample, EOMI controls and low BMI group. Individuals with extreme phenotypes (N=170) are from high (N=85) and low LDL (N=85) group.

2.2.2 Reference Panel Construction A reference panel of 2163 individuals (including the 1692 African Americans used in this study and 471 European Americans) was constructed. All of the 2163 individuals have both Genome-wide association study (GWAS; Affymetrix 6.0) genotypes and whole exome sequencing data. When combining the two sources of data, a total of 375024 bi-allelic autosomal SNPs with minor allele count $\geq 4$ (in the 2163 reference panel subjects) did not overlap with the 70205 GWAS SNPs. There were 10130 SNPs that overlapped between ESP and the 70205 GWAS markers. SNPs with concordance <95% were removed (65 SNPs). For overlapping SNPs that passed this concordance filter, GWAS genotype was retained for consistency with the target individuals. A total of 1077164 autosomal SNPs were included in the reference panel. These 1077164 markers were phased across all 2163 samples using BEAGLE v3.3.1 (Browning and Yu, 2009).

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2.2.4 The 1000 Genomes Project (1000G) The 1000 Genomes Phase1 data were downloaded from http://www.sph.umich.edu/csg/ylh/mach/download/1000G.2012-03-14.html. Details regarding the generation of the data can be found in the Phase 1 article (The 1000 Genomes Project Consortium, 2012).

2.3 Target African Americans

2.3.1 GWAS Data All of the 1661 target African Americans in this study were genotyped using the Affymetrix 6.0 genotyping platform as part of the WHI SNP Health Association Resource study. Before phasing and imputation, we removed Affymetrix 6.0 SNPs with genotype call rates <90%, or Hardy-Weinberg exact test (Wigginton et al., 2005) $P<10^{-6}$ or MAF <1%. QC details were described previously (Auer et al., 2012; Reiner et al., 2011).

2.3.2 MetaGobchips data All of the 1661 target African Americans in this study were also genotyped using the MetaBiosip (Voight et al., 2012) in an attempt to generalize genetic effects across racial groups by the
WHI Population Architecture using Genomics and Epidemiology (PAGE) study. Standard QC was performed, including removal of markers with genotype call rate <95% or Hardy–Weinberg $P<10^{-6}$, as well as exclusion of individuals who showed excess heterozygosity, were part of an apparent first-degree relative pair, or were ancestry outliers as determined by Eigensoft (Price et al., 2006). Details can be found in the PAGE Metabochip article (Buyske et al., 2012).

Genotypes at the Metabochip SNPs were not used for imputation but rather used for assessment of imputation quality. In total 5035 markers, which were on Metabochip, in 1000G and in ESP, but not on Affymetrix 6.0, were used for imputation quality assessment.

2.3.3 Overlap with ESP African Americans African Americans present in ESP were not included as target. In other words, individuals in the reference ESP and the target were mutually exclusive. In addition, we removed any target with PLINK (Purcell et al., 2007) estimated identity-by-descent (IBD) ≥ 0.2 with any reference individual such that our final target set did not contain any apparent first-degree relative with the reference ESP.

2.3.4 Imputation using IMPUTE2 In the main text, unless otherwise specified, we present results using minimac for imputation. Supplementary Figure S5 and Supplementary Table S7 showed that our recommendation of ESP alone or concatenation of ESP with 1000G (ESP_U_1000G) over 1000G still held when IMPUTE2 was used for imputation. We note that in the main text, our recommendation against IMPUTE2’s two panel mode (option 3: ESP + 1000G) was confounded by software/method choice: ESP alone or ESP_U_1000G using minimac performed better than IMPUTE2’s ESP + 1000G, but when using IMPUTE2 for all, ESP alone or ESP_U_1000G performed similarly as ESP + 1000G.

3 RESULTS

3.1 Comparison of imputation quality between ESP-based and 1000G-based imputation

We first performed imputation, using either ESP or 1000G as reference, into 1661 African Americans in the WHI study (the ‘target’ sample) who were genotyped by both the Affymetrix 6.0 (Auer et al., 2012) and the Illumina Metabochip array (Buyske et al., 2012; Liu et al., 2012). We used MaCH (Li et al., 2010), a hidden Markov model that leverages linkage disequilibrium information among samples of unrelated individuals, to pre-phase the 1661 WHI African Americans at the Affymetrix 6.0 markers. The ESP reference panel was built from 1692 African Americans with genotypes from both the Affymetrix 6.0 platform and whole genome sequencing, deep exome sequencing and SNP array genotyping. Second, ESP African Americans (~50% also from WHI, detailed in Materials and Methods) were better matched to the ‘target’ WHI African Americans for ancestry than were the samples in the 1000G panel, which were pooled from several populations of European, African and African American ancestry.

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Fig. 1. Comparison of dosage $r^2$ between ESP-based and 1000G-based imputation. The $x$-axis is the proportion of SNPs that were removed based on elevated Rsq threshold (QC). The $y$-axis is the mean dosage $r^2$ (squared Pearson correlation between imputed dosages and experimental genotypes).
As expected, better quality imputation using the ESP panel produces a larger number of well-imputed rare coding variants than using the 1000G panel (Rsq $\geq 0.6$ for MAF $\leq 0.5\%$; detailed in Table 1). For example, the number of well-imputed variants was 2.28, 2.83, and 1.54 times greater than that from 1000G for MAF $\leq 0.2$, 0.2–0.5 and 0.5–1%, respectively (Table 1). The boost in imputation quality as well as in the number of well-imputed markers is expected to enhance power for testing association with phenotypic traits. For example, out of the eight novel blood trait associated variants reported in Auer et al. (Auer et al., 2012), two are not in 1000G but ESP only (Supplementary Table S3).

### 3.2 Impact of imputation reference panel constructed from subjects selected based on extreme phenotypes

Many subjects sequenced in ESP were selected on the basis of phenotypic extremes or disease status (detailed in Materials and Methods), an approach that has been shown to increase power for association testing of the specific phenotype (Barnett et al., 2013; Guey et al., 2011; Kryukov et al., 2009). To our knowledge, the consequences of such a design for developing an imputation reference panel have not been previously evaluated. To this end, we constructed two ESP-derived reference panels: ‘ESP.extreme’ and ‘ESP.normal’ each of size $H = 853 \times 2$. The former included 254 African Americans from LDL cholesterol extremes, 247 from blood pressure extremes, 40 stroke cases, 39 early onset MI (EOMI) cases and 273 with extremely high BMI. The latter included 85 samples with high LDL, 85 with low LDL and 683 from the ‘middle’ of the phenotype distributions. We observed no loss of imputation quality using the ‘Extreme’ panel. (Fig. 3 and Supplementary Table S4).

### 3.3 Alternative options to use or combine ESP and 1000G reference panels

Although our results suggested that the ESP panel led to substantially improved imputation accuracy of rare coding variants compared with the 1000G panel, the combination of the two panels could potentially result in even better performance than either one individually. We considered the following four options. The default option, Option 0, was to select a single panel a priori based on reference panel size, marker density and ancestry match. In this case, Option 0 would be the ESP reference panel alone, as it contains more haplotypes (3384 over 2184 in 1000G), greater marker density in exons and a better ancestry match with the target African Americans. Option 1 was to first impute using each panel separately, and then for each marker to select the one with higher Rsq. Option 2 was to impute using a concatenated panel of the two (ESP_U_1000G). Option 3 was to impute using IMPUTE2, which allows two separate reference panels (ESP + 1000G).

The best option among the four was the concatenation of the two panels (Option 2) with ESP alone (Option 0), a close second best. For example, the average dosage $r^2$ increased by 1.8%, 2.3% and 1.5%, respectively, for markers with MAF $<0.2$, 0.2–0.5 and 0.5–1% using Option 2 over Option 0 (Supplementary Fig. S2 and Supplementary Table S5). We observed no noticeable gains using Option 1 compared with

### Table 1. Number and percentage of well-imputed exonic variants

<table>
<thead>
<tr>
<th>MAF</th>
<th>Number (%) of well-imputed markers</th>
<th>ESP:1000G ratio (Number of well-imputed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.2%</td>
<td>17,606 (31.8)</td>
<td>7713 (3.0)</td>
</tr>
<tr>
<td>0.2–0.5%</td>
<td>26,255 (70.0)</td>
<td>9283 (26.9)</td>
</tr>
<tr>
<td>0.5–1%</td>
<td>21,377 (92.1)</td>
<td>13,882 (62.9)</td>
</tr>
<tr>
<td>1–3%</td>
<td>29,784 (96.7)</td>
<td>26,466 (90.7)</td>
</tr>
<tr>
<td>3–5%</td>
<td>11,490 (96.9)</td>
<td>11,043 (96.0)</td>
</tr>
<tr>
<td>5–50%</td>
<td>40,500 (98.0)</td>
<td>39,849 (96.3)</td>
</tr>
</tbody>
</table>

*aWell-imputed is defined such that the average Rsq of the QC+ markers within each MAF category is >0.8.*
mean dosage were removed based on elevated Rsq threshold (QC). The ESP with Option 0. For example, dosage panels (Option 3), led to decreased imputation quality compared because higher Rsq does not guarantee better imputation quality. we would not recommend using Option 1, the Rsq-based selection, (Supplementary Fig. S3 and Supplementary Table S6). Therefore, using IMPUTE2 in Materials and Methods, Supplementary Fig. 4D I S C U S S I O N

Although we recommend the concatenation of ESP and 1000G, we observed only modest gains in imputation quality by combining the two. Previous studies suggest that these gains may depend in part on the ethnic make-up of the study subjects (Browning and Yu, 2009) and whether 1000G data add substantial haplotype diversity. These gains should be weighed against the logistical challenges of combining data from multiple sources to avoid batch effects (e.g. mismatched strands, consistent marker naming schemes or systematic differences in geno-
type calling, QC or phasing).

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of WHI investigators can be found at: https://cleo.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Short%20List.pdf.

The PAGE coordinating center (U01HG004801-01) provides assistance with study design, phenotype harmonization, SNP selection and annotation, data cleaning, data management, integration and dissemination, and general study coordination. Genotype calling, genotype QC and statistical analyses are also performed by the coordinating center for some PAGE studies. The National Institute of Mental Health also contributes to the support for the coordinating center.

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**REFERENCES**


