Title
Sequencing and imputation in GWAS: Cost-effective strategies to increase power and genomic coverage across diverse populations.

Permalink
https://escholarship.org/uc/item/309835jj

Journal
Genetic epidemiology, 44(6)

ISSN
0741-0395

Authors
Quick, Corbin
Anugu, Pramod
Musani, Solomon
et al.

Publication Date
2020-09-01

DOI
10.1002/gepi.22326

Peer reviewed
Sequencing and imputation in GWAS: Cost-effective strategies to increase power and genomic coverage across diverse populations

Corbin Quick1 | Pramod Anugu2 | Solomon Musani2 | Scott T. Weiss3,4,5 | Esteban G. Burchard6,7 | Marquitta J. White6 | Kevin L. Keys6 | Francesco Cucca8,9 | Carlo Sidore8 | Michael Boehnke1 | Christian Fuchsberger1,10,11

1Department of Biostatistics and Center for Statistical Genetics, University of Michigan School of Public Health, Ann Arbor, Michigan
2University of Mississippi Medical Center, Jackson, Mississippi
3Harvard Medical School, Boston, Massachusetts
4Channing Department of Network Medicine, Brigham and Women’s Hospital, Boston, California
5Partners HealthCare Personalized Medicine, Boston, Massachusetts
6Department of Medicine, University of California San Francisco, San Francisco, California
7Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California
8Istituto di Ricerca Genetica e Biomedica (IRGB), CNR, Monserrato, Italy
9Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy
10Department of Genetics and Pharmacology, Institute of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria
11Institute for Biomedicine, Eurac Research, Affiliated Institute of the University of Lübeck, Bolzano, Italy

Abstract
A key aim for current genome-wide association studies (GWAS) is to interrogate the full spectrum of genetic variation underlying human traits, including rare variants, across populations. Deep whole-genome sequencing is the gold standard to fully capture genetic variation, but remains prohibitively expensive for large sample sizes. Array genotyping interrogates a sparser set of variants, which can be used as a scaffold for genotype imputation to capture a wider set of variants. However, imputation quality depends crucially on reference panel size and genetic distance from the target population. Here, we consider sequencing a subset of GWAS participants and imputing the rest using a reference panel that includes both sequenced GWAS participants and an external reference panel. We investigate how imputation quality and GWAS power are affected.
power are affected by the number of participants sequenced for admixed populations (African and Latino Americans) and European population isolates (Sardinians and Finns), and identify powerful, cost-effective GWAS designs given current sequencing and array costs. For populations that are well-represented in existing reference panels, we find that array genotyping alone is cost-effective and well-powered to detect common- and rare-variant associations. For poorly represented populations, sequencing a subset of participants is often most cost-effective, and can substantially increase imputation quality and GWAS power.

**KEYWORDS**
genotype imputation, genotyping, GWAS, rare variants, sequencing, study design, WGS

## 1 INTRODUCTION

Genome-wide association studies (GWAS) have detected thousands of common genetic variants associated with hundreds of complex diseases and traits (MacArthur et al., 2016). A key aim for the next wave of GWAS is to interrogate the full spectrum of genetic variation underlying human genetic traits, including rare (minor allele frequency [MAF] < 0.5%) variants, across a wide range of human populations. Detecting association at rare variants requires both more comprehensive genomic coverage and sufficient sample size. Deep whole genome sequencing (WGS) is the gold standard method for capturing rare variation; however, even in the era of the $1,000 genome, large WGS association studies remain prohibitively expensive. Genotype imputation has been a mainstay of GWAS, providing increased genomic coverage from inexpensive array-based genotype call sets. While initial imputation studies only surveyed common variants (e.g., Scott et al., 2007), larger and more diverse reference panels now enable more accurate and comprehensive imputation of rare and low-frequency (0.5% < MAF < 5%) variants across a wide range of populations (e.g., Mahajan, Wessel, et al., 2018).

Imputation algorithms model haplotypes in the study sample as mosaics of haplotypes in a reference panel (e.g., from the International HapMap Project Consortium, 2010 or 1000 Genomes Project Consortium, 2015) to predict genotypes at untyped variants (Li, Willer, Sanna, & Abecasis, 2009). By increasing genomic coverage and accuracy, imputation increases statistical power to detect association, enables more complete meta-analysis of results from multiple studies, and facilitates the identification of causal variants through genetic fine-mapping (Das et al., 2016; Li et al., 2009). Imputation coverage and accuracy depend crucially on the size of the reference panel and the genetic distance between reference and target populations (Li et al., 2009; Roshyara & Scholz, 2015). The largest current broadly available reference panels, for example, from the Haplotype Reference Consortium (HRC; McCarthy et al., 2016) and UK10K project (UK10K; UK10K Consortium, 2015), include tens of thousands of predominantly European individuals. These panels provide near complete imputation of genetic variation down to MAF ~ 0.1% for many European populations, but lower imputation quality for non-European and admixed populations and population isolates, particularly for rare and low-frequency variants (Deelen et al., 2014; Pistis et al., 2015; Zhou et al., 2017). The 1000 Genomes Project and HapMap panels include individuals from diverse worldwide populations, but provide more limited imputation coverage and accuracy due to their smaller sample sizes.

Capturing rare variation across diverse populations is crucial to detect population differences in genetic risk factors, accurately predict genetic risk, and identify causal variants and biological mechanisms through trans-ethnic fine-mapping (Kichaev & Pasaniuc, 2015; Popejoy & Fullerton, 2016). Population-matched or multiethnic reference panels can improve imputation quality and coverage for rare variants in GWAS of diverse populations (Ahmad et al., 2017; Deelen et al., 2014; Lencz et al., 2018; Pistis et al., 2015; Van Leeuwen et al., 2015; Zhou et al., 2017); this approach has enabled discovery of novel loci and refinement of association signals for multiple populations and complex traits (Auer & Lettre, 2015; Holm et al., 2011; Pistis et al., 2015).

Here, we consider an approach in which a subset of study participants is whole genome sequenced and the rest are array-genotyped and imputed using an augmented reference panel that comprises the sequenced participants and individuals from an external reference panel (Hu, Li, Auer, & Lin, 2015; Zeggini, 2011). This hybrid sequencing-and-imputation strategy provides more comprehensive coverage than array genotyping alone, and is less costly than WGS the entire sample. We and others have used this strategy (Fuchsberger et al., 2016; Sidore et al., 2015; Steinthorsdottir...
et al., 2014; Van Leeuwen et al., 2015), but no analysis of coverage, power, and cost-effectiveness has been carried out to date. Here, we assess how imputation coverage and power to detect association vary across genotyping arrays and as a functions of the number of population-matched individuals sequenced and included in the reference panel for two admixed populations (African Americans and Latino Americans) and two European population isolates (Sardinians and Finns) to identify powerful and cost-effective GWAS strategies in these populations. We also describe an interactive web-based tool to assist researchers in the design and planning of their own GWAS.

2 | METHODS

We first describe WGS data sources used in our analysis. Next, we describe imputation strategies, and outline procedures and imputation quality metrics to compare these strategies. Finally, we present a novel method to estimate power for the sequencing-only, imputation-only, and sequencing-and-imputation strategies. For ease of presentation, we assume a dichotomous trait and a multiplicative disease model, although our findings generalize easily to continuous traits and other genetic models.

2.1 | Data resources

We used WGS data on 11,920 individuals to assess imputation quality across reference panel configurations and genotyping arrays for admixed populations and population isolates. For our analysis of admixed populations, we used WGS data on 3,412 African Americans (participants from the Jackson Heart Study [phs000964]) and 2,068 Latino Americans (participants of Puerto Rican and Mexican descent from the GALA II study [phs000920] and Costa Rican descent from the Genetic Epidemiology of Asthma in Costa Rica and CAMP studies [phs000921]) in the National Heart, Lung, and Blood Institute (NHLBI) Trans-Omics for Precision Medicine (TOPMed) WGS program. For our analysis of isolated populations, we used WGS data on 2,995 Finns (participants of the GoT2D, 1KGP, SISu, and Kuusamo studies) and 3,445 Sardinians (participants of the SardiNIA study) in the HRC (EGAS00001001710).

2.2 | Procedures to evaluate imputation coverage and accuracy

We considered three imputation strategies: (a) using sequenced study participants as a study-specific reference panel, (b) using an external reference panel alone (for this comparison, the HRC or HRC subset excluding individuals from the target population), and (c) using an augmented panel that comprises sequenced study participants and an external panel.

For African Americans, who are underrepresented in the current version 1.1 of the HRC, we constructed population-specific and HRC-augmented reference panels with 0–2,000 African Americans. For Latino Americans, we used the same approach but restricted the study-specific panel size to <1,500 due to the more limited available sample of sequenced Latino American individuals. For Finns and Sardinians, which are present in the HRC, we constructed augmented reference panels that comprised the 29,470 non-Finnish or 29,020 non-Sardinian individuals in the HRC together with 0–2,000 Finns or Sardinians from the HRC.

For each population, each imputation strategy, and each of three commonly used genotyping arrays (Table 1), we used sequence-based genotype calls at marker variants present on the array as a scaffold for imputation using Minimac3, masking the remaining sequence-based genotype calls (Das et al., 2016). Results using other commonly used imputation software, for example, IMPUTE2 (Howie, Donnelly, & Marchini, 2009) and Beagle (Browning & Browning, 2016), are expected to be highly similar to those from Minimac3 based on previous studies comparing performance and consistency across genotype imputation software (e.g., Browning & Browning, 2016; Roshyara, Horn, Kirsten, Ahnert, & Scholz, 2016; Shi et al., 2018).

We then compared the imputed genotype dosages to the true (masked) genotypes to estimate (a) imputation $r^2$, the squared Pearson correlation between true genotype and imputed dosage, and (b) imputation coverage, the proportion of variants with imputation $r^2 > 0.3$ and minor allele count (MAC) $\geq 5$ (the MAC threshold used

| TABLE 1 | Genotyping arrays used for comparisons |
| --- | --- | --- | --- |
| Array | No. marker variants | List cost per sample Illumina (2018) |
| Illumina Infinium Core | 307 K | $49 |
| Illumina Infinium OmniExpress | 710 K | $94 |
| Illumina Infinium Omni2.5 | 2.5 M | $172 |
null hypothesis of no association.

The asymptotic distribution of \( \hat{r}_{nj} \) is normal with mean 0 and variance 1, where \( \hat{r}_{nj} \) is the squared correlation between imputed dosages and true genotypes, and \( \eta \) is an effect size parameter which is equal to 0 under the null hypothesis of no association. The form of \( \eta \) depends on the association model (e.g., additive, dominant, multiplicative), RR or odds ratio, MAF, and population prevalence and, for binary traits, the case–control ratio. Under an arbitrary association model for binary traits, we can write

\[
\eta = \frac{2(p_{\text{case}} - p_{\text{control}})}{\sqrt{(1 + s)(v_{\text{case}} + \frac{1}{s}v_{\text{control}}) + 4(p_{\text{case}} - p_{\text{control}})^2}}
\]

where \( p_{\text{case}} \) and \( p_{\text{control}} \) are the alternate allele frequencies in the disease-positive and disease-negative populations, \( v_{\text{case}} \) and \( v_{\text{control}} \) are the variances of genotypes in the disease-positive and disease-negative populations, and \( s \) is the GWAS case–control ratio.

To estimate power while accounting for variability in imputation \( r^2 \) and the possibility that a variant is not imputable, we average empirical imputation \( r^2 \) values and MACs across variants from experiments with real data described in the previous section. Specifically, we estimate power to detect association when \( n \) individuals are sequenced and \( m \) are genotyped and imputed as

\[
\text{Power}(m, n) = \frac{1}{\sum w_j^{\text{MAF}} w_j^{\text{PS}}} \sum w_j^{\text{MAF}} w_j^{\text{PS}} C_{nj} \int_{-z_{\alpha/2}}^{z_{\alpha/2}} \phi(u - \eta \sqrt{n + r_{nj}^2 m}) \, du,
\]

where \( \phi(u) = e^{-u^2/2\pi} \) is the standard normal density function, \( z_{\alpha/2} \) is the \( \alpha \)-level significance threshold, \( r_{nj}^2 \) is the imputation \( r^2 \) value for the \( j \)th variant, \( C_{nj} = I(MAC_{\text{panel}} \geq 5, r_{nj}^2 \geq 0.3) \) is an indicator equal to 1 if the \( j \)th variant was imputable and 0 otherwise, and \( MAC_{\text{panel}} \) is the reference panel MAC for the \( j \)th variant when the \( n \) sequenced individuals from the target population were included in the reference panel.

We define the first weight term \( w_j^{\text{MAF}} = p_{N\text{GWAS}}(\hat{p}_j)/\hat{p}_j \), where \( N \) is the total number of samples used in our analysis for the given population (e.g., \( N = 3,412 \) for African Americans), \( \hat{p}_j \) is the sample MAC for the \( j \)th variant in the total sample, \( p_{N\text{GWAS}}(x) \) is the probability of observing sample MAC = \( x \) in a sample of size \( N \) given the specified association model. For example, in a GWAS with sample size \( N \) and case–control ratio \( s \), the sample MAC (which is equal to 2\( \hat{p} \), where \( \hat{p} \) is the sample MAF) is approximately Poisson distributed with mean 2\( N (s p_{\text{case}} + p_{\text{control}})/(s + 1) \), where \( p_{\text{case}} = p/(1 + p(\gamma - 1)) \) and \( p_{\text{control}} = (p - K p_{\text{case}})/(1 - K) \) for a variant with population MAF \( p \) and RR \( \gamma \) for a disease with prevalence \( K \). This weighting approach adjusts for differences between the empirical distribution of MACs across variants in real data, and the theoretical MAC distribution for a variant with the specified MAF, effect size, prevalence in a GWAS with sample size \( N \) and case–control ratio \( s \).

The second weighting term \( w_j^{\text{PS}} \) accounts for the probability that a variant with the specified population MAF \( p \) is population-specific (monomorphic outside the target population), and is defined

\[
w_j^{\text{PS}} = \begin{cases} 
\hat{P}_{\text{PS}}(p)/\hat{P}_{\text{PS}}(\hat{p}_j), & \text{variant } j \text{ is population-specific}, \\
[1 - \hat{P}_{\text{PS}}(p)]/[1 - \hat{P}_{\text{PS}}(\hat{p}_j)], & \text{otherwise},
\end{cases}
\]

where \( \hat{P}_{\text{PS}}(x) \) is the fraction of variants that are population-specific among variants with MAF = \( x \) in the reference panel.
target population. This adjustment factor ensures that the weight assigned to population-specific variants in power calculations reflects the probability that a variant with the specified population MAF \( p \) is population-specific.

3 | RESULTS

First, we compare strategies to improve imputation using study-specific WGS data for African Americans, Latino Americans, Sardinians, and Finns. Next, we assess the effects of genotyping array on imputation quality and coverage for each population and reference panel. We then use these results to estimate statistical power to detect association as a function of study-specific panel size, number of participants imputed, external reference panel, and genotyping array. Finally, we identify cost-effective study designs by comparing statistical power and total experimental (sequencing and genotyping) costs for sequencing-only, imputation-only, and sequencing-and-imputation GWAS designs for each population and genotyping array.

3.1 | Strategies to improve imputation using Study-Specific WGS data

We compared imputation \( r^2 \) and coverage (proportion of variants with imputation \( r^2 > 0.3 \) and reference \( \text{MAC} \geq 5 \)) for three imputation strategies: (a) using an external reference panel (the HRC or HRC subset) alone, (b) using an augmented reference panel that combines the study-specific and external panels, and (c) using a study-specific reference panel alone.

The external panel alone (HRC for Latino Americans and African Americans, and HRC subset that excludes individuals from the target population for Finns and Sardinians) provided 96% imputation coverage for MAF \( \geq 0.25\% \) variants (where MAF is calculated separately within each population) for Finns, 84% coverage for Sardinians, 86% coverage for Latino Americans, and 77% coverage for African Americans (Figure 1, top row). The relatively lower coverage for African Americans is expected since the HRC consists primarily of Central and Northern Europeans, who are genetically closer to Finns and Sardinians, and includes relatively few Africans or African Americans. Despite the small number of Latino or Native Americans included in the HRC, imputation coverage was slightly higher for Latino Americans than for Sardinians. This may reflect the high degree of European admixture in many Latino American populations (Bryc et al., 2010), and the abundance of population-specific rare and low-frequency variants in the Sardinian population (Sidore et al., 2015).

Augmenting an external reference panel with even a relatively small number of sequenced individuals substantially increased coverage, particularly for African Americans and Sardinians, and for variants with lower MAF. For example, augmenting the external panel with 500 sequenced individuals from the study population provided 96% coverage for African Americans and 77% coverage for Sardinians (Figure 1, middle row). Augmenting an external reference panel with 500 sequenced individuals increased coverage for Sardinians and for variants with lower MAF to 86% coverage for Sardinians and 77% coverage for African Americans (Figure 1, bottom row).

![Figure 1](image_url)

**Figure 1** Imputation quality by population and genotyping array. Imputation coverage (upper panels) and mean imputation \( r^2 \) (lower panels) as functions of the number of population-matched individuals included in augmented reference panels (number sequenced, \( x \)-axis). Here and elsewhere, MAF is calculated separately within each population. MAF, minor allele frequency.
population improved overall imputation coverage for MAF = 0.25–0.5% variants by 4% for Finns, 9% for Latino Americans, 16% for African Americans, and 23% for Sardinians genotyped using the OmniExpress relative to the external panel alone (Figure 1). Similarly, augmenting the external reference panel with even 200 individuals increased imputation coverage for MAF = 0.1–0.25% variants by 3%, 4%, 6%, and 10% relative to the external panel alone for Finns, Latino Americans, African Americans, and Sardinians, respectively.

With 2,000 individuals from the target population (or 1,500 for Latino Americans), population-specific panels provided roughly equivalent imputation $r^2$ compared to augmented panels (Figure S1A); however, augmented panels provided higher imputation coverage overall for low MAF variants (Figure S1B). For example, augmented panels with 2,000 individuals from the target population (or 1,500 for Latino Americans) provided 86%, 80%, 79%, and 86% coverage for 0.1–0.25% MAF variants for Finns, Latino Americans, African Americans, and Sardinians respectively, whereas population-specific panels alone provided 72%, 51%, 78%, and 72% coverage using the Omni Express array. However, imputation coverage for variants with MAF > 0.25% differed by <1% between augmented and population-specific panels with 2,000 individuals from the target population (or 1,500 for Latino Americans) for all populations and genotyping arrays. When a smaller number (less than 500) of individuals from the target population are sequenced, augmented reference panels provided substantially higher imputation coverage and $r^2$ than population-specific panels alone. For example, augmented panels with 500 individuals from the target population provided 90%, 85%, 65%, and 85% coverage for 0.25–0.5% MAF variants for Finns, Latino Americans, African Americans, and Sardinians respectively, whereas population-specific panels of 500 individuals provided ≤30% coverage using the Omni Express array.

Even very rare variants (MAF = 0.1–0.25%) attained high coverage across all populations given a sufficient number of population-matched individuals in the reference panel. For example, attaining ≥70% imputation coverage for MAF = 0.1–0.25% variants required a study-specific panel of ≥1,800 individuals for African Americans, 1,000 for Latino Americans, 700 for Sardinians, and 0 for Finns using the OmniExpress. These increases in imputation coverage primarily reflect increasing numbers of population-specific variants captured in the reference panel, which are absent from or present in low copy number in the external panel.

### 3.2 Imputation coverage and quality across genotyping arrays

Imputation coverage was generally similar for the OmniExpress and Omni2.5 arrays, but consistently lower for the less dense Core array. Coverage differed by <7% between the OmniExpress and Omni2.5 across all MAF bins, populations, and reference panels, whereas the Core provided up to 24% lower coverage than the Omni2.5 (Figure 1, upper panels). Imputation coverage was more heterogeneous across arrays for populations with greater genetic distance from the external reference panel (e.g., African Americans and the HRC panel), particularly with smaller (or absent) study-specific panels. Because we used the same reference panels for each genotyping array, differences in imputation coverage between arrays are solely due to differences in the proportion of variants that attained imputation $r^2 ≥ 0.3$. Imputation $r^2$ varied more across genotyping arrays than did imputation coverage (Figure 1, lower vs. upper panels); however, the magnitude of differences in imputation $r^2$ between arrays was still generally modest, particularly for the Finns and Sardinians.

### 3.3 Powerful and cost-effective strategies for GWAS across populations

We compared the cost-effectiveness of sequencing-only, imputation-only, and sequencing-and-imputation strategies by analyzing statistical power to detect association as a function of numbers of study participants sequenced and imputed, genotyping array, and reference panel across a range of genetic models. Here, we define the most cost-effective strategy as either (a) minimizing total experimental (sequencing and genotyping) cost while attaining power at or above a given threshold, or equivalently (b) maximizing power while maintaining cost no greater than a specified constraint.

The cost-effectiveness of sequencing a subset of study participants varied greatly across populations. For Finns, imputation-only designs were most powerful to detect association and adding sequenced individuals increased power only minimally, even for low-frequency and rare variants. For Sardinians, Latino Americans, and African Americans, sequencing a subset of study participants was optimal, and often achieved substantially greater power than imputation-only or sequencing-only studies. For example, a GWAS of African Americans with equal numbers of cases and controls in which 400 participants are sequenced and 11,100 are imputed using the Illumina Infinium Infinium Core array has 90% power to detect a risk
variant with MAF = 0.5% and RR = 4 for a disease with prevalence 1%, whereas an imputation-only GWAS with the same total cost (19,250 participants) has only 68% power (Figure 2). Even for populations in which optimal sequencing-and-imputation designs had substantially greater power than imputation-only, the optimal number to sequence was often modest. For example, only 210 participants are sequenced under the optimal design using the Illumina OmniExpress to attain 80% power in the previous example (Figure 3). This is expected because even a relatively small study-specific panel can substantially increase imputation coverage (Figure 1, upper panels).

### 3.4 Denser genotyping arrays versus sequencing: Which is more cost-effective to increase power?

Imputation coverage and power to detect association can be increased by using denser genotyping arrays, which provide a more informative framework for imputation, or by sequencing population-matched individuals and augmenting the reference panel. We assessed the cost-effectiveness of these two strategies by comparing power to detect association across genotyping arrays for study designs that have the same total cost assuming $1,000 for WGS and current list prices for genotyping arrays (Table 1). As expected, the optimal number of participants sequenced to maximize power given fixed total cost generally decreased with increasing array density. For example, the optimal number sequenced to maximize power to detect association was 500, 300, and 90 for the Infinium Core, OmniExpress, and Omni2.5, respectively for Sardinians given total sequencing and genotyping budget of $2 M for a risk variant with RR = 2, MAF = 1%, and disease prevalence 1%. Power to detect association under the optimal design given a fixed total cost was generally greater for sparser arrays; in the previous example, power under the optimal design was 98%, 91%, and 55% for the Infinium Core, OmniExpress, Omni2.5.

We also compared optimal designs to attain power above a given threshold at minimum total cost across genotyping arrays based on the per-sample array genotyping costs reported in Table 1. Generally, sparser arrays were more cost-effective (reached the power threshold with lower total cost) than dense arrays. In fact, the sparsest genotyping array in our analysis, the Infinium Core, was most cost-effective across all disease models and populations apart from African Americans, for whom the Infinium OmniExpress was most cost-effective for some rare-variant disease models. This last result is unsurprising given the substantial difference in imputation coverage between the Infinium Core and Omni arrays for African Americans (Figure 1).

![Figure 2](image.png)

**Figure 2** Power and optimal design by population and genotyping array. Power to detect association for case-control studies with equal numbers of cases and controls as a function of sequenced subsample size (x-axis) and imputed subsample size (y-axis) for a variant with MAF 0.5% and RR 4 for a disease with prevalence 1%. Axes are scaled to reflect costs of genotyping arrays (Table 1) and sequencing ($1 K per sample). Dashed diagonal lines indicate study designs with the same total cost, given by $y = a - bx$, where $a = (Total\text{cost}/Array\text{cost})$ and $b = (Sequencing\text{cost})/(Array\text{cost})$. Circled points indicate optimal study designs, which attain the indicated power level at minimum total experimental cost (or, maximize power at the indicated total experimental cost), shown only for optimal designs with total genotyping cost ≤ $2 M ($1.5 M for Latino Americans). MAF, minor allele frequency; RR, relative risk.
sequencing/genotyping. Under these assumptions, we found that denser arrays are generally less cost-effective than sparser arrays; of course, denser arrays provide higher imputation coverage given a fixed GWAS sample size.

3.5 | Optimal study design as a function of MAF and effect size

Power to detect association under a given study design depends on MAF, effect size (relative risk [RR] or odds ratio), and population prevalence Sham and Purcell (2014). These parameters also influence the relative cost-effectiveness of sequencing and imputation. While common variants can be accurately imputed with small reference panels, large population-matched reference panels are needed to capture rare (population-specific) variants. In Figure 3, we illustrate the impact of sequencing on statistical power for two combinations of MAF and effect size in each of the four study populations.

The optimal percentage of study participants sequenced to attain ≥80% power decreases with increasing effect size magnitude. This is expected, since the expected number of risk alleles captured in the reference panel increases with effect size magnitude.

4 | DISCUSSION

While the cost of genome sequencing has fallen dramatically (Sham & Purcell, 2014), large genome sequencing studies remain prohibitively expensive. Large imputation reference panels are now enabling accurate imputation of even very rare variants (MAF > 0.001; Mahajan, Taliun, et al., 2018; McCarthy et al., 2016; Zhou et al., 2017), making imputation-based GWAS viable and cost-effective for detecting associations across much of the allele frequency spectrum. For populations with limited reference panel data, we have shown that sequencing a subset of study participants can substantially increase imputation coverage and accuracy, particularly for rare and population-specific variants, at a fraction of the cost of sequencing the entire study cohort. Our results also suggest that it is almost always advantageous to augment existing reference panels, except when the study-specific sequenced panel is large or the target population has high genetic distance from the external panel.
Complementary sequencing-and-imputation GWAS strategies have been applied to refine association signals and discover novel associations for several populations and complex traits (Auer & Lettre, 2015; Holm et al., 2011; Pistis et al., 2015). While most sequencing-and-imputation studies to date have been carried out in European isolated populations, our results suggest that this strategy can also be powerful and cost-effective for admixed and non-European populations. In addition to increasing genomic coverage and power to detect association for the study itself, sequencing a subset of study participants provides a data resource that can be used to enhance imputation in future studies of the same or related populations so long as the sequence data can be shared.

Directly augmenting an existing reference panel with study-specific sequence data is not always feasible due to technical, logistical, and privacy constraints. However, we and others have found that the distributed reference panel approach (separately imputing with two or more reference panels and combining the results) provides nearly equivalent imputation quality (Figure S2). Thus, study-specific WGS data can be used to improve imputation even when directly augmenting an external panel is not feasible.

We assumed that sequenced participants are randomly selected from the overall set of GWAS participants. When array-based genotype data are collected before sequencing, samples could be strategically selected to maximize imputation quality or minimize redundancy in the reference panel. Indeed, strategic selection of individuals to be sequenced for an imputation reference panel has been explored in several previous studies. For example, Zhang, Zhan, Rosenberg, and Zollner (2013) proposed a selection procedure to maximize phylogenetic diversity within the reference panel; related methods were proposed by Kang and Marjoram (2012) and Pascaniuc et al. (2010). Based on results of these previous studies, we expect gains from strategic selection to be relatively modest for the data sets considered in our manuscript. Our results based on random selection can be viewed as a lower bound for the improvement in imputation accuracy that could be gained by strategic selection of individuals for a reference panel of the same size. We also note that existing methods are intended for selecting individuals for an internal reference panel, and do not directly apply to the setting where an external reference panel, for which individual-level genotype data are inaccessible, is used in addition to the study’s own internal reference panel. Extensions of these methods that account for external data are certainly conceivable and worthy of future research.

While large reference panels enable accurate imputation across a wide range of the allele frequency spectrum (McCarthy et al., 2016; Zhou et al., 2017), the extent of genetic variation that can be captured through imputation is limited relative to WGS. For example, de novo mutations cannot be imputed regardless of
reference panel size. This is particularly salient for monogenic disorders; for example, over 80% of achondroplasia cases occur from recurrent de novo mutations in FGFR3 (Bellus et al., 1995). Thus, imputation may be unable to detect causative alleles for traits with extreme genetic architectures, even with very large reference panels.

As increasingly large and diverse sequencing projects are conducted, larger and more diverse reference panels will become available. In the design and planning of GWAS, it may be prudent to consider resources under development and pending release in addition to resources that are currently available. More broadly, our analysis highlights the utility of collaboration and coordination across institutions for effective study design and resource allocation. For example, the optimal design to maximize power in an individual study does not necessarily maximize meta-analysis power across multiple studies of the same trait and population.

Our analysis of cost-effectiveness and optimal design depends crucially on the relative per-sample costs of sequencing and array genotyping. Both sequencing and array genotyping costs have fallen markedly in recent years, and are likely to continue to do so. Depending on the relative rates of change, cost-effectiveness and optimal design also may change. In addition, the cost of participant recruitment and DNA sample collection may alter the relative cost-effectiveness of sequencing and genotyping. Finally, our cost-effectiveness analysis assumes that sample size is unconstrained; this may not apply for small populations or rare diseases.

While our results are illustrative, investigators may wish to explore questions of the relative cost-effectiveness of sequencing and array genotyping strategies in the context of their own study and relevant assumptions about population, reference panels, and sequencing and array genotyping costs. To enable this exploration, we have developed a flexible, easy-to-use tool, Analysis of Power for Sequencing and Imputation Studies (APSIS), which is open source and freely available at http://github.com/corbing/APSIS.

5 | CONCLUSIONS

Here, we assessed the genomic coverage, statistical power, and cost-effectiveness of sequencing and imputation-based designs for GWAS in four populations across a range of genetic models. We developed a novel method to account for available reference haplotype data in power calculations using empirical data, which can be applied to inform GWAS planning and design. For European populations that are well-represented in current reference panels, our results suggest that imputation-based GWAS is cost-effective and well-powered to detect both common- and rare-variant associations. For populations with limited representation in current reference panels, we found that sequencing a subset of study participants can substantially increase genomic coverage and power to detect association, particularly for rare and population-specific variants. Our results also suggest that larger and more diverse reference panels will be important to facilitate array-based GWAS in global populations.

ACKNOWLEDGMENTS

The authors acknowledge support from NIH grant R01 HG009976 (MB), the Austrian Science Fund (FWF) grant J-3401 (CF), NIH contracts N01-AG-1-2109 and HHSN271201100005C (FC), and The Fondazione di Sardegna, Prot. U1301.2015/AI.1157. BE Prat. 2015-1651 (FC). A complete list of NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium coinvestigators is provided in Supporting Information Materials. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

TOPMed

Whole genome sequencing (WGS) for the Trans-Omics in Precision Medicine (TOPMed) program was supported by the National Heart, Lung and Blood Institute (NHLBI). WGS for NHLBI TOPMed: Genes-Environments and Admixture and Latino Americans (GALA II) Study (phs000920.v1.p1) was performed at the New York Genome Center (3R01HL117004-01S3). WGS for NHLBI TOPMed: The Jackson Heart Study (phs000964.v1.p1) was performed at the University of Washington Northwest Genomics Center (HHSN268201100037C). WGS for NHLBI TOPMed: The Genetic Epidemiology of Asthma in Costa Rica (phs000988.v1.p1) was performed at the University of Washington Northwest Genomics Center (3R37HL066289-13S1). Centralized read mapping and genotype calling, along with variant quality metrics and filtering were provided by the TOPMed Informatics Research Center (3R01HL117626-02S1). Phenotype harmonization, data management, sample-identity QC, and general study coordination, were provided by the TOPMed Data Coordinating Center (3R01HL120393-02S1). We gratefully acknowledge the studies and participants who provided biological samples and data for TOPMed.

JACKSON HEART STUDY

The Jackson Heart Study (JHS) is supported and conducted in collaboration with Jackson State University...
THE GENETICS OF ASTHMA IN COSTA RICA PROJECT
The Genetics of Asthma in Costa Rica project is supported by R-37 HL066289 (PI-Scott T. Weiss) and P01 HL132825-01 (PI-Scott T. Weiss) both from the National Heart, Lung, and Blood Institute. The investigators wish to acknowledge the families who contributed data to this study and to Juan C. Celedon, MD, DPH, Lydia Avila MD, and Manuel Soto-Queros, MD who led the data collection team in Costa Rica.

GENES-ENVIRONMENTS AND ADMIXTURE IN LATINO AMERICANS STUDY
The Genes-Environments and Admixture in Latino Americans (GALA II) Study and E. G. B. are supported by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, the Harry Wen. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II, the National Heart, Lung, and Blood Institute (NHLBI) R01HL117004, R01HL128439, R01HL133516, X01HL134589, the National Institute of Environmental Health Sciences R01ES015794, R21ES24844, the National Institute on Minority Health and Health Disparities (NIMHD) P60MD006902, R01HL135156, the Tobacco-Related Disease Research Program under Award Number 24RT-0025. The authors wish to acknowledge the following GALA II coinvestigators for subject recruitment, sample processing and quality control: Celeste Eng, Scott Huntsman, MSc, Donglei Hu, PhD, Angel CY Mak, MPhil, PhD, Shannon Thyne, MD, Harold J. Farber, MD, MSPH, Pedro C. Avila, MD, Denise Serebrisky, MD, William Rodriguez-Cintron, MD, Jose R. Rodriguez-Santana, MD, Rajesh Kumar, MD, Luisa N. Borrell, DDS, PhD, Emerita Brigino-Buenaventura, MD, Adam Davis, MA, MPH, Michael A. LeNoir, MD, Kelley Meade, MD, Saunak Sen, PhD and Fred Lurmann, MS. The authors also wish to thank the staffs and participants contributed to the GALA II study.

SISu AND KUUSAMO
The SISu and Kuusamo data set comprise of selected study samples from the FINRISK and Health2000 (H2000) studies. FINRISK study has been primarily funded by budgetary funds of THL (National Institute for Health and Welfare) with important additional funding from the Academy of Finland (grant number 139635 for VS) and from the Finnish Foundation for Cardiovascular Research. The H2000 study was funded by the National Institute for Health and Welfare (THL), the Finnish Centre for Pensions (ETK), the Social Insurance Institution of Finland (KELA), the Local Government Pensions Institution (KEVA) and other organizations listed on the website of the survey (http://www.terveys2000.fi). Both studies are grateful to the THL DNA laboratory for its skillful work to produce the DNA samples used in this study. We thank the Sanger Institute sequencing facilities for whole genome sequencing of the Kuusamo subset. A. P. and S. R. are supported by the Academy of Finland (grant no. 251704, 286500, 293404 to A. P., and 251217, 285380 to S. R.). Juselius Foundation, Finnish Foundation for Cardiovascular Research, NordForsk eScience NIASC (grant no. 62721) and Biocentrum Helsinki (to SR).

GOT2D
Funding for this study was provided by: The Academy of Finland (139635); Action on Hearing Loss (UK; G51); The Ahokas Foundation; The Andrea and Charles Bronfman Philanthropies; The British Heart Foundation (SP/04/002); The Canadian Institutes of Health Research; The Estonian Government (IUT24-6, IUT20-60); The European Commission (ENGAGE: HEALTH-F4-2007-201413); The European Community's Seventh Framework Programme (FP7/2007–2013; EpiMigrant, 279143); The European Regional Development Fund (3.2.0304.11-0312); The European Research Council (ADG20110310#293574); The European Union (EX-GENESIS); The Finnish Diabetes Research Foundation; The Finnish Foundation for Cardiovascular Research; The Finnish Medical Society; The Folkhälsans Research Foundation (Finland); The Fonds de la Recherche en Santé du Québec (Canada); The Foundation for Life and Health in Finland; The German Federal Ministry of Education and Research (BMBF) The German Federal Ministry of Health (BMG); The German Center for Diabetes Research (DZD); Helmholtz Zentrum München (German Research Center for Environmental Health), which is supported by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria; The Helsinki University Central Hospital Research Foundation; The KG Jebsen Foundation (Norway); The Knut och Alice Wallenberg Foundation (HHSN268201300049C and HHSN268201300050C), Tougaloo College (HHSN268201300048C), and the University of Mississippi Medical Center (HHSN268201300046C and HHSN268201300047C) contracts from the National Heart, Lung, and Blood Institute (NHLBI) and the National Institute for Minority Health and Health Disparities (NIMHD). The authors also wish to thank the staffs and participants of the JHS.
DATA AVAILABILITY STATEMENT
Summary data describing imputation quality and coverage for each population and genotyping array are provided as a part of the APSIS (Analysis of Power for Sequencing-and-Imputation Studies) software tool, which is open-source and freely available at http://github.com/corbinq/APSIS

ORCID
Christian Fuchsberger http://orcid.org/0000-0002-5918-8947

REFERENCES
Ahmad, M., Sinha, A., Ghosh, S., Kumar, V., Davila, S., Yajnik, C. S., & Chandak, G. R. (2017). Inclusion of population-specific reference panel from india to the 1000 genomes phase 3 panel improves imputation accuracy. Scientific Reports, 7(1), 6733. https://doi.org/10.1038/s41598-017-06905-6
Auer, P. L., & Lettre, G. (2015). Rare variant association studies: Considerations, challenges and opportunities. Genome Medicine, 7(1), 16. https://doi.org/10.1186/s13073-015-0138-2
Bellus, G. A., Hefferson, T. W., de Luna, R. O., Hecht, J. T., Horton, W. A., Machado, M., ... Francomano, C. A. (1995). Achondroplasia is defined by recurrent G380R mutations of FGFR3. American Journal of Human Genetics, 56(2), 368.
Browning, B. L., & Browning, S. R. (2016). Genotype imputation with millions of reference samples. Genotype Imputation with Millions of Reference Samples, 98(1), 116–126.
Bryc, K., Velez, C., Karafet, T., Moreno-Estrada, A., Reynolds, A., Auton, A., ... Ostrer, H. (2010). Genome-wide patterns of population structure and admixture among Hispanic/Latino populations. Proceedings of the National Academy of Sciences of the United States of America, 107, 8954–8961.
Das, S., Forer, L., Schonherr, S., Sidore, C., Locke, A. E., Kwong, A., ... Fuchsberger, C. (2016). Next-generation genotype imputation service and methods. Nature Genetics, 48(10), 1284–1287. https://doi.org/10.1038/ng.3656
Deelen, P., Menelaou, A., Van Leeuwen, E. M., Kanterakis, A., ... Estrada, K. (2014). Improved imputation quality of low-frequency and rare variants in European samples using the 'Genome of The Netherlands'. European Journal of Human Genetics, 22(11), 1321–1326.
Fuchsberger, C., Flannick, J., Teslovich, T. M., Mahajan, A., Agarwala, V., Gaulton, K. J., ... McCarthy, M. I. (2016). The genetic architecture of type 2 diabetes. Nature, 536(7614), 41–47. https://doi.org/10.1038/nature18642
Genomes Project Consortium. (2015). A global reference for human genetic variation. Nature, 526(7571), 68–74.
Holm, H., Gudbjartsson, D. F., Sulem, P., Masson, G., ... Gylfason, A. (2011). A rare variant in MYH6 is associated with high risk of sick sinus syndrome. Nature Genetics, 43(4), 316–320.
Howie, B. N., Donnelly, P., & Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLOS Genetics, 5(6), e1000529.
Hu, Y. J., Li, Y., Auer, P. L., & Lin, D. Y. (2015). Integrative analysis of sequencing and array genotype data for discovering disease associations with rare mutations. Proceedings of the National Academy of Sciences of the United States of America, 112(4), 1019–1024. https://doi.org/10.1073/pnas.1406143112
Illumina (2018). Microarray kits for genotyping and epigenetic analysis. Retrieved from https://www.illumina.com/products/by-type/microarray-kits.html
International HapMap 3 Consortium. (2010). Integrating common and rare genetic variation in diverse human populations. Nature, 467(7311), 52–58.
Kang, C. J., & Marjoram, P. (2012). A sample selection strategy for next-generation sequencing. Genetic Epidemiology, 36(7), 696–709. https://doi.org/10.1002/gepi.21664
Kichaev, G., & Pasaniuc, B. (2015). Leveraging functional-annotation data in trans-ethnic fine-mapping studies. American Journal of Human Genetics, 97(2), 260–271. https://doi.org/10.1016/j.ajhg.2015.06.007
Lencz, T., Yu, J., Palmer, C., Carmi, S., Ben-Avraham, D., Barzilai, N., ... Pe'er, I. (2018). High-depth whole genome sequencing of a large population-specific reference panel: Enhancing sensitivity, accuracy, and imputation. Human Genetics, 137, 343–355.
McCarthy, S., Das, S., Kretzschmar, W., Delaneau, O., Wood, A. R., Roshyara, N. R., & Scholz, M. (2015). Impact of genetic similarity on imputation performance. *Human Heredity, 83*(3), 107–116. https://doi.org/10.1159/000489758

Sidore, C., Busonero, F., Maschio, A., Porcu, E., Naitza, S., Zoledziewska, M., ... Abecasis, G. R. (2015). Genome sequencing elucidates Sardinian genetic architecture and augments association analyses for lipid and blood inflammatory markers. *Nature Genetics, 47*(11), 1272–1281. https://doi.org/10.1038/ng.3368

Steinhorsdottir, V., Thorleifsson, G., Sulem, P., Helgason, H., Grarup, N., Sigurdsson, A., ... Stefansson, K. (2014). Identification of low-frequency and rare sequence variants associated with elevated or reduced risk of type 2 diabetes. *Nature Genetics, 46*(3), 294–298. https://doi.org/10.1038/ng.2882

UK10K Consortium. (2015). The UK10K project identifies rare variants in health and disease. *Nature, 526*(7571), 82–90.

Van Leeuwen, E. M., Kanterakis, A., Deelen, P., Kattenberg, M. V., Abdellaoui, A., Hofman, A., ... van Schaik, B. D. (2015). Population-specific genotype imputations using minimac or IMPUTE2. *Nature Protocols, 10*(9), 1285.

Zeggini, E. (2011). Next-generation association studies for complex traits. *Nature Genetics, 43*(4), 287–288. https://doi.org/10.1038/ng.90411-287

Zhang, P., Zhan, X., Rosenberg, N. A., & Zollner, S. (2013). Genotype imputation reference panel selection using maximal phylogenetic diversity. *Genetics, 195*(2), 319–330. https://doi.org/10.1534/genetics.113.154591

Zhou, W., Fritsche, L. G., Das, S., Zhang, H., Nielsen, J. B., Holmen, O. L., ... Hveem, K. (2017). Improving power of association tests using multiple sets of imputed genotypes from distributed reference panels. *Genetic Epidemiology, 41*(8), 744–755.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Quick C, Anugu P, Musani S, et al. Sequencing and imputation in GWAS: Cost-effective strategies to increase power and genomic coverage across diverse populations. *Genetic Epidemiology*. 2020;44:537–549. https://doi.org/10.1002/gepi.22326