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RESEARCH ARTICLE

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

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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

Michael Boehnke and Christian Fuchsberger jointly supervised this work.

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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 wellrepresented 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 finemapping (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 1Genotyping arrays used forcomparisons

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

by the HRC panel, McCarthy et al., 2016) in the reference panel.

2.3 | Estimating power to detect association using empirical imputation quality data

When sequenced individuals are included in the reference panel, power calculations should account for the interdependence between imputation r^2 and the number of participants sequenced n, and for the possibility that the variant is not imputable (absent in the reference panel or not imputed due to insufficient MAC, or filtered before association analysis due to imputation r^2 falling below a given threshold). While common variant associations are likely to be captured by LD proxy single nucleotide polymorphisms (SNPs) even when the causal variant is not directly genotyped or imputed, rare variant associations are much less likely to be captured by proxy SNPs (Montpetit et al., 2006). Here, we assume that power to detect association for variants that are not imputable is zero. This assumption affects power calculations almost exclusively for rare variants, since common variants are almost uniformly imputable with large reference panels (Das et al., 2016; McCarthy et al., 2016).

We assume that the *n* participants who are sequenced are randomly subsampled from the overall sample of n + m study participants, and that test statistics are calculated separately for the sequenced and imputed subsamples and combined using the effective sample size weighted meta-analysis test statistic $Z_{nm} =$ $c_{nm}^{1/2}Z_n^{seq} + (1 - c_{nm})^{1/2}Z_m^{imp}$, where $c_{nm} = n/(n + r^2m)$. The asymptotic distribution of $Z_{nm} - \eta \sqrt{n} + r^2 m$ is normal with mean 0 and variance 1, where r^2 is the squared correlation between imputed dosages and true genotypes, and η is an effect size parameter which is equal to 0 under the null hypothesis of no association. The form of η 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

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

where p_{case} and $p_{control}$ are the alternate allele frequencies in the disease-positive and disease-negative populations, v_{case} and $v_{control}$ 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

$$\widehat{Power}(m,n) = \frac{1}{\sum_{j} w_{j}^{MAF} w_{j}^{PS}} \sum_{j} w_{j}^{MAF} w_{j}^{PS} C_{nj} \int_{-z_{1-\alpha/2}}^{z_{1-\alpha/2}} \phi \left(u - \eta \sqrt{n + r_{nj}^{2}m}\right) du,$$

where $\phi(u) = e^{-\frac{u^2}{2}}/\sqrt{2\pi}$ is the standard normal density function, $z_{1-\alpha/2}$ is the α -level significance threshold, r_{nj}^2 is the imputation r^2 value for the *j*th variant, $C_{nj} = I(MAC_{nj}^{panel} \ge 5, r_{nj}^2 \ge 0.3)$ is an indicator equal to 1 if the *j*th variant was imputable and 0 otherwise, and MAC_{nj}^{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 $w_i^{MAF} = P_N^{GWAS}(\hat{p}_i)/\hat{P}_N(\hat{p}_i)$, 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}_i is the sample MAF for the *j*th variant in the total sample, $\hat{P}_N(x)$ is the proportion of variants with MAF = x, and $P_N^{GWAS}(x)$ is the probability of observing sample MAF = 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 $2N\hat{p}$, where \hat{p} is the sample MAF) is approximately Poisson distributed with mean $2N(sp_{case} + p_{control})/(s + 1)$, where $p_{case} =$ $p\gamma/[1 + p(\gamma - 1)]$ and $p_{control} = (p - Kp_{case})/(1 - K)$ for a variant with population MAF p and RR γ 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^{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_i^{PS}

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

where $\hat{P}_{PS}(x)$ is the fraction of variants that are population-specific among variants with MAF = x in the

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 sequencingonly, 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 MAC ≥ 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 studyspecific 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 populationspecific 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



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

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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 populationspecific 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 populationspecific 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 $r^2 \ge 0.3$. 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.

Powerful and cost-effective 3.3 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 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 costeffectiveness 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). Importantly, our analysis assumes (a) a direct trade-off between the GWAS sample size and sequencing/array genotyping costs, and (b) no additional costs per GWAS sample other than



Power to detect association for a variant with RR=4, MAF=0.5%, and disease prevalence 1%

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 = (Totalcost/(Arraycost) and b = (Sequencingcost)/(Arraycost). 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 $\leq 2 M$ (\$1.5 M for Latino Americans). MAF, minor allele frequency; RR, relative risk



FIGURE 3 Power as a function of MAF and effect size. Statistical power (*y*-axis) to detect a rare large-effect variant (MAF = 0.25%, RR = 3; top row) and common modest-effect variant (MAF = 5%, RR = 1.3; bottom row) for a disease with prevalence 1% as a function of the number of participants array-genotyped and imputed (*x*-axis) when 0, 500, or 2,000 participants are sequenced and included in an augmented reference panel. The number of participants sequenced has a far greater impact on statistical power for the rare variant association. Importantly, statistical power is bounded above by the probability that the variant is imputable ($r^2 > 0.3$ and reference MAC ≥ 5), causing power to asymptote below 1 as a function of the number of imputed participants (e.g., upper-left panel). MAC, minor allele count; 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 costeffectiveness 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 \geq 80% power to detect association at minimum total cost increases with decreasing MAF (Figure 4). This is expected, since larger reference panels are needed to capture variants with lower frequency. Finally, the optimal percentage of study participants sequenced to attain \geq 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.



FIGURE 4 Optimal design as a function of minor allele frequency and effect size. Percentage of participants sequenced (*x*-axis) and total sample size (*y*-axis) under optimal designs to attain statistical power \geq 80% for rare and common variants across two effect size values for each of the four study populations using the Infinium Core array. Here, effect size refers to the χ^2 NCP for single-variant association tests given perfect genotype accuracy, which is defined as η^2 in Section 2. RR values corresponding to each combination of MAF and NCP are indicated in the far-right panel (for Sardinians). With NCP held constant, differences in optimal design for different MAF values are solely due to differences in imputation coverage and quality across the MAF spectrum. MAF, minor allele frequency; NCP, noncentrality parameter; RR, relative risk

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 Pasaniuc 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.

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GENES-ENVIRONMENTS AND ADMIXTURE IN LATINO AMERICANS STUDY

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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/corbing/APSIS

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SUPPORTING INFORMATION

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

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