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Genome-wide association studies in ancestrally diverse populations: opportunities, methods, pitfalls, and recommendations.

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Supplemental Material:

Includes Supplemental Methods I-VII and Supplemental Tables S1–S4.

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Abstract

Genome-wide association studies (GWAS) have focused primarily on populations of European descent, but it is essential that diverse populations become better represented. Increasing diversity among study participants will advance our understanding of genetic architecture in all populations and ensure that genetic research is broadly applicable. To facilitate and promote research in multi-ancestry and admixed cohorts, we outline key methodological considerations and highlight opportunities, challenges, solutions, and areas in need of development. Despite the perception that analyzing genetic data from diverse populations is difficult, it is scientifically and ethically imperative, and there is an expanding analytical toolbox to do it well.

Keywords

GWAS; ancestry; diversity; cross-ancestry; trans-ancestry; trans-ethnic; population genetics; admixed populations; psychiatry; complex disease

A disproportionate majority (>78%) of participants in published genome-wide association studies (**GWAS**) are of European descent (Popejoy and Fullerton, 2016; Sirugo et al., 2019), with 71.8% of these individuals having been recruited from just three countries: the United States, the United Kingdom, and Iceland (Mills and Rahal, 2019). Studies of major psychiatric disorders are no exception, having focused largely on populations of European ancestry (Figure 1). Conducting GWAS in individuals of European ancestry was a practical starting point given the availability of samples and limited funding, genotyping technologies,

and analytic methods. However, there is now widespread acknowledgement of the need for more diverse samples and for improved analytic methods. Broadening diversity of studied populations will improve the effectiveness of genomic medicine by expanding the scope of known human genomic variation and bolstering our understanding of disease etiology. Consensus in the field points to many benefits of increased representation of more diverse populations for locus discovery, fine-mapping, polygenic risk scores, and addressing existing health disparities (Duncan et al., 2018; Hindorff et al., 2018a; Lam et al., 2018; Martin et al., 2019; Walters et al., 2018).

With increasing representation of global populations in GWAS, there is an opportunity for advanced methods development and a need for consensus “best practices” for analyzing the emerging complex datasets. Here, we provide background on the scientific and ethical importance of including underrepresented groups in genetics research and offer guidance for whole-genome analysis of ancestrally diverse study cohorts. We summarize currently available resources and make recommendations for avoiding practices that could lead to false-positives, loss of statistical power, or misinterpretation of results. Because this primer represents a collaborative product of the Cross-Population Special Interest Group of the Psychiatric Genomics Consortium (PGC) (<https://www.med.unc.edu/pgc/cross-population/>), we have framed our discussion within the context of psychiatric genetics. Nevertheless, the points and recommendations outlined herein are applicable to any complex biomedical phenotype.

Genetic ancestry is estimated from DNA and provides information about shared demographic history at the population level. Individuals with similar ancestral origins have shared genomic signatures due to migration of common ancestors, mutations and recombination, genetic drift, and natural selection. These processes yield differences in allele frequencies and linkage disequilibrium (**LD**) patterns across populations (Barrett and Cardon, 2006; International HapMap Consortium, 2005) that must be properly addressed to avoid false positive genetic findings. In addition to ancestral diversity, the current lack of racial and ethnic diversity, which are related but distinct from ancestry (see Box 1), hinder the development of more complete etiological models (Banda et al., 2015; Medina-Gomez et al., 2015; Race, Ethnicity, and Genetics Working Group, 2005). In complex disease research, race and ethnicity can provide information about social, cultural, and environmental factors that affect risk for disease, including having a lived experience of social injustice. Given that these socio-cultural measures are often inappropriately used as a proxy for genetic ancestry, researchers and clinicians should be careful to distinguish among them in order to tease apart specific biological, environmental, and social determinants of health.

Inclusion of diverse study participants in genomics research has yielded important scientific insights for a range of human traits and diseases. The resolution of fine-mapping improves through cross-ancestry analysis (Wojcik et al., 2019). Estimates of effect-sizes derived from cohorts of diverse ancestries tend to be more accurate than from those of a single ancestry (Li and Keating, 2014). Genetic risk prediction attenuates with increasing divergence between the discovery and target populations, indicating that polygenic risk scores (**PRS**) based on Eurocentric GWAS are not equally predictive when applied to non-European populations (Duncan et al., 2018; Martin et al., 2019). Conversely, constructing individual-

level scores from cross-ancestry meta-analysis results improves overall prediction (Grinde et al., 2019; Márquez-Luna et al., 2017).

Besides the strong scientific justifications for broader inclusion, there are important ethical, legal, and public health reasons for bolstering diversity in genomics (Hindorff et al., 2018b). Understanding how genetic risk and social inequities interact to influence disparities in disease risk and outcomes will be critical to improving public health.

Moreover, while integration of genomics into healthcare has the potential to improve disease prediction and optimize treatments, a lack of diversity will limit the utility of precision medicine efforts: individuals of non-European descent are more likely to receive ambiguous test results from genetic screening (e.g., variants of unknown or uncertain significance) (Petrovski and Goldstein, 2016) and false positive diagnoses (Manrai et al., 2016). There is also a higher chance of false negative diagnoses in individuals from ancestral backgrounds that are not well represented in clinical databases, due to missing information about additional disease-causing variants currently not on testing panels (Minster et al., 2016; Moltke et al., 2014; Wheeler et al., 2017). Similarly, the potential benefits of pharmacogenetics cannot be fully realized until there is equitable representation across ancestries, as some therapeutics may be more effective and/or safer in certain populations because of differences in allele frequency, effect size, and penetrance of variants associated with drug metabolism (Roden et al., 2011). Here, we provide an accessible framework for analyzing these data, while acknowledging that there are several important methodological areas in need of further development. Key terminology is bolded and defined in Box 2.

Methodological Considerations

In the analysis of multi-ancestry datasets, a significant concern is false positive genetic signals due to inflated test statistics from **population stratification**, which occurs when disease prevalence and allelic frequency differences are correlated within or between study cohorts (Marchini et al., 2004). Two typical strategies exist for addressing this challenge while analyzing samples from multiple major/admixed populations: (1) Empirically assign samples to major continental and/or admixed populations using genome-wide data, analyze each population separately, and conduct cross-ancestry meta-analysis (*stratified meta-analysis approach*), and (2) analyze samples from multiple populations together, most commonly with a mixed model (*joint mixed model approach*). The choice between these approaches is perhaps the most broadly impactful decision currently facing analysts of genome-wide data from multiple populations since it impacts methodological considerations in all analysis steps from quality control, to reference alignment in imputation, to association model, to the suitability of results for secondary analyses. We highlight elements of GWAS where the choice between the stratified meta-analysis and joint mixed model approaches is particularly salient. Figure 2 shows a general workflow for each approach.

Genotyping Technologies

Most genome-wide DNA microarrays were designed for individuals of European ancestry. The differences in LD structure and allele frequency among populations can lead to significantly worse coverage for other ancestry groups. For example, at imputation accuracy

$r^2 > 0.8$, the Affymetrix UK Biobank array covers 84% of the variants that have minor allele frequencies (MAF) $> 1\%$ in samples of European ancestry but only 46% of those for samples of African ancestry (Nelson et al., 2017). The large genetic diversity in African populations means that a larger number of variants are needed on arrays in order to provide similar coverage as in other populations (Barrett and Cardon, 2006). To address this issue, some groups, such as China Kadoorie Biobank (Chen et al., 2011), have designed population-specific arrays. Multi-ancestry arrays, such as the Multi-Ethnic Global Array (MEGA), Global Screening Array (GSA), and the H3Africa array (Mulder et al., 2018) were designed based on panels with more diverse ancestries, and are therefore recommended. An alternative strategy is to sequence whole genomes; low-depth sequencing has received recent attention for application in diverse samples due to cost-effectiveness and higher coverage with acceptable error rates ((Gilly et al., 2018; Peterson et al., 2017a); see *Rare Variants*).

Quality Control

Quality control (QC) of GWAS data aims to remove low quality data and technical artifacts in order to reduce the risk of false positive associations. In diverse ancestry cohorts, the main issue is that many common QC criteria assume the sample comes from a homogeneous population. Applying standard QC procedures without adjustment for population structure leads to the erroneous removal of too many variants and samples from minority subgroups and admixed samples, reducing statistical power.

QC criteria that are dependent on population allele frequencies can generally be adapted for application in diverse cohorts by either stratifying the cohort into major populations prior to filtering (the stratified meta-analysis approach) or by adjusting the QC measure to allow for varying allele frequencies (the joint mixed model approach; see Figure 2). For example, individuals are often removed based on excess autosomal heterozygosity, as a potential indication of sample contamination, but the standard heterozygosity statistic assumes each variant's expected allele frequency is constant across individuals. In diverse cohorts, regressing this heterozygosity statistic on principal components prior to identifying outliers can avoid excessive exclusions of individuals from subgroups in the cohort. Step-by-step considerations for common QC criteria, including sample QC workflows for the stratified meta-analysis and joint mixed model approaches, are given in Supplemental Methods I (see also Supplemental Table S2, Supplemental Figure 1). In addition to these pre-imputation QC steps, post-imputation QC steps should also consider ancestry (see *Imputation*).

Inferring Population Structure

Estimating the genetic population structure of a cohort typically serves two primary goals in GWAS: 1) to characterize the ancestral diversity of the cohort as a descriptive measure and 2) to provide a quantitative estimate of population structure that can be used in QC and in GWAS association models to reduce the risk of false positives. We focus here on use for description and QC, and later discuss methods for controlling for population structure (see *Genome-wide Association*).

For cohorts with diverse ancestral backgrounds, we can estimate population structure based on genome-wide data. Currently the most common tool for estimating continuous population

structure is principal component analysis (**PCA**); a listing of other approaches is included in Supplemental Methods II. PCA is a statistical method for reducing the complexity of high-dimensional data (e.g., thousands of measured variants across the genome) into orthogonal axes (principal components, PCs) that explain the largest fraction of variability in the data. The spread of data across these axes provides a visual guide to sub-structure among samples; when data points are estimated from each individual's genetic markers, the PCs illustrate population structure. These PCs can be computed within the cohort, or can be estimated from an external reference (e.g., The 1000 Genomes Project (1KGP); (Sudmant et al., 2015)) and the GWAS sample can be projected onto the PC axes to allow comparison with the ancestries of known reference populations (Peterson et al., 2017b). However, the latter approach can be limited by the number and diversity of populations represented on the **reference panel**, highlighting the need for many additional diverse population references to be generated. PCs may also be used to control for ancestry structure in other QC metrics (see *Quality Control* and Supplemental Table S2).

This sample-wide estimation and visualization of genetic ancestry can be used to empirically assign genetically similar samples into more homogenous groups. This assignment is necessary for the *stratified meta-analysis approach* to GWAS of diverse cohorts, and is intended to reduce the risk of false positive genetic signals due to inflated test statistics from **population stratification**. Assigning samples to more homogeneous groups for analysis reduces stratification by limiting the degree of population structure remaining in the sample. Samples with a specific admixture can be assigned into their own major ancestral group, instead of being excluded from the analysis or forced into other ancestry groups, provided there are adequate numbers of individuals in the sample with comparable admixed backgrounds. However, it is often the case that genomic outliers (which tend to be from under-represented or admixed backgrounds) might need to be excluded if there is an insufficient number of other individuals who fall into a similar cluster. These assignment methods will not provide - and are not intended to provide - detailed ancestral background information for each individual. Rather, they provide a working solution to reduce false positives due to population stratification (Hellwege et al., 2017). We stress that sample group assignment and identifying appropriate reference population panels can be difficult, particularly for admixed ancestry, thus requiring careful inspection of data and methods (Medina-Gomez et al., 2015).

Imputation and Population Reference Panels

GWAS arrays genotype a portion of common variation. Genotype imputation is a cost-effective computational approach for inferring genotypes or genotype probabilities at variants that have not been directly genotyped on GWAS arrays, based on comparisons to genetic data from external reference samples. Imputation increases the number of markers available for association testing and can harmonize cohorts genotyped on different arrays for meta-analysis.

Imputation accuracy relies on having an appropriate reference panel that includes **haplotypes** from the population studied. Matching alleles and allele frequencies in the study cohort with reference panels as part of pre-imputation QC also relies on using reference data

from a matched ancestral background. Reference panels with better coverage of haplotypes from the population of the genotyped cohort will yield a greater number of well-imputed variants for GWAS, especially among lower frequency variants (Ahmad et al., 2017; Howie et al., 2012). Table 1 lists major imputation panels that are currently publicly available. We note that although many ongoing projects are aiming at more diverse populations (Supplemental Table S3), additional efforts in more populations are needed to expand the diversity of imputation reference panels (Kelleher et al., 2018).

Current imputation methods are summarized in Supplemental Methods III. Joint imputation using the largest applicable reference panel is expected to perform at least as well as subsetting that reference panel to match the target population (Ahmad et al., 2017; Howie et al., 2012), possibly due to maintaining a larger sample size for phasing. Use of the same reference panel for all cohorts also avoids potential confounding with varying imputation quality. However, it may be necessary to consider imputation quality separately within subsets of individuals even if the samples are jointly imputed since imputation accuracy for a variant may vary widely across individuals of different ancestries.

Genome-wide Association

The core of GWAS analysis is testing the association between each variant and a target phenotype. As noted, a primary consideration for association testing in diverse cohorts is whether to stratify samples into major population groups or to analyze the full cohort jointly (assuming imputation was also done jointly). In either case, the major concern is proper control of population stratification to ensure that observed associations reflect genetic effects of each locus rather than correlations with ancestry.

Joint analysis using a mixed model approach is attractive because all participants are included irrespective of ancestry. Ideally, mixed model approaches control for population stratification by modelling distant relatedness between individuals due to ancestry (Sul et al., 2018; Wojcik et al., 2019). Several implementations exist and some are listed in Supplementary Methods Section IV and Supplementary Table S4. Mixed models may yield greater statistical power, both through increased sample size and by controlling for the variance explained by the genetic relatedness between individuals (i.e., a random effects component; (Loh et al., 2018)). However, there is evidence that basic mixed models may not fully control for population structure in diverse cohorts, especially if there is an environmental component to phenotypic associations with ancestry beyond the modelled genetic relatedness (Conomos et al., 2018; Heckerman et al., 2016; Zhang and Pan, 2015). Non-genetic factors such as environmental exposures may be correlated with ancestry due to a shared local environment (familial or community effects) or due to the relationship between ancestry and socio-cultural factors such as race and ethnicity. More methodological development is needed before mixed models or other strategies for joint GWAS of a diverse cohort can be confidently recommended as robust.

When stratifying by population backgrounds, covariates such as PCs should still be used to correct for population stratification. Conventional linear or logistic regression with these covariates can be used for association testing as long as QC included exclusion of related individuals; mixed models or other alternatives with PC covariates may be applied in family-

based samples stratified by ancestry (Walters et al., 2018). Computing these PCs separately *within* each ancestry subset instead of the full study ensures better control for residual structure specific to that subset (e.g., fine structure, genotyping/technical artifacts), but at the cost of potentially reduced control for stratification related to population structure shared across subsets (Patterson et al. 2006). For analyses of admixed or multi-ancestry cohorts, PCs may still be included in the regression but additional covariates may be required to control for stratification that is not linear in PCA space (Conomos et al., 2018; Heckerman et al., 2016; Zhang and Pan, 2015). For example, race and ethnicity are often correlated with socio-economic status and other environmental risk factors for disease. Self-reported ethnicity or other variables that capture trait heterogeneity on the basis of socio-cultural factors may also be appropriate to consider as covariates in those instances (Banda et al., 2015; Medina-Gomez et al., 2015). Directly controlling for local ancestry tracts in variant-level association analyses may further improve power and reduce false positives in admixed samples (Li & Keating 2014).

The meta-analysis approach, combining separate analyses of samples stratified by similar genetic background, currently has several pragmatic advantages. First, computational pipelines developed for single-ancestry analyses can be used for each cohort. Separate analysis also naturally provides ancestry-specific results, which may be valuable for secondary analyses including PRS (Bulik-Sullivan et al., 2015; Lam et al., 2018). Reduced environmental variability within a subset may also improve power. On the other hand, splitting each cohort may be challenging due to continuous gradients of admixture or small sample sizes within an ancestry group. This loss of information from excluding individuals from diverse genomic backgrounds is a missed opportunity for discovery and validation of GWAS findings, and thus additional approaches need to be developed and leveraged.

Meta-analysis of GWAS Summary Statistics

Traditional meta-analytic approaches for GWAS rely on fixed-effects models that assume a given variant has the same true marginal effect size across all studies. This assumption is likely to be violated in meta-analyses across diverse cohorts. Even when the causal genetic effect of a variant is constant across populations, as seems common in cross-ancestry GWAS to date (Huang et al., 2017; Lam et al., 2018), marginal effect sizes may show heterogeneity when LD structures are different. Further heterogeneity across cohorts from different populations may arise due to differences in genetic background (e.g., gene \times gene interactions) and/or environmental context (e.g., gene \times environment interactions), as well as differences in study design (e.g., imputation artifacts, phenotyping). As a result, it is generally appropriate to model this cross-cohort heterogeneity in meta-analysis by using a random effects or trans-ancestral meta-analysis model (Supplementary Methods Section 5, Supplementary Table S4).

Fine-mapping

A trait-associated locus from GWAS typically implicates a large genomic region with many variants of similar significance. This set may contain a few causal variants, while the association of other variants is driven by their LD with the causal one(s). Fine-mapping refines GWAS loci to a smaller set of likely causal variants to facilitate interpretation and

follow-up studies (Schaid et al., 2018). Fine-mapping studies in samples of European ancestry have made important advances, with some loci resolved even to single-variant resolution (Huang et al., 2017; Mahajan et al., 2018). Because fine-mapping assumes the causal variant(s) have been observed, non-European populations face a unique challenge due to the lack of representation of many variants as a result of incomplete sampling from these populations, suboptimal chip design, and limited imputation performance.

Combining samples across ancestries has an advantage for fine-mapping: the LD patterns that differ across populations can improve the resolution, assuming that many causal variants are shared across populations, which has been shown true for some traits, including schizophrenia (Lam et al., 2018; Marigorta and Navarro, 2013; Wojcik et al., 2019). Non-causal variants tagging the causal variants have marginal different effects across populations if LD is different, thus allowing the causal variant to be distinguished from non-causal variants. Furthermore, in certain populations (e.g., African), LD blocks are generally smaller, so fewer non-causal variants will tag the causal variants, improving the resolution of fine-mapping (International HapMap Consortium, 2005; Schaid et al., 2018).

Most fine-mapping algorithms (Huang et al., 2017; Schaid et al., 2018) can be applied to samples from multiple ancestries combined through meta-analysis. However, this strategy does not take full advantage of genomic diversity across populations. An alternate Bayesian fine-mapping strategy (Lam et al., 2018) more precisely mapped the schizophrenia genetic associations through explicitly modeling diversity in LD between East Asian and European samples. This approach works on a presumption that the causal variants and their effect sizes are identical across populations, which is not always true. PAINTOR (Kichaev and Pasaniuc, 2015) relaxes this presumption by allowing the effect size to vary across populations, although the causal variant still needs to be the same. Fine-mapping methods will benefit from continued development that appropriately models LD and relies on fewer assumptions.

Polygenic Risk Scores in Diverse Populations

PRS are individual-level estimates of the relative genetic contribution to a phenotype, computed for each genotyped individual in a target sample based on GWAS results from a discovery sample. PRS are useful for validating GWAS results in external cohorts and have the potential to provide individualized risk prediction from genetic data (Khera et al., 2018; Martin et al., 2019). The predictive value of **PRS** profiling depends both on the statistical power of the discovery (training) dataset— specifically, enrichment in the genome-wide distribution of association test statistics that is attributable to aggregate, additive genetic effects — and the relevant characteristics of the target (testing) dataset.

In particular, PRS accuracy is also a function of recent human demographic history, such that a greater proportion of phenotypic variance is explainable in target populations that are genetically more similar to the population studied in the discovery GWAS. Stated another way, with increasing genetic “distance” between the discovery and target datasets, there is often attenuation of polygenic predictive value. Furthermore, because most participants in large GWAS have been broadly European (Figure 1), most PRS currently perform best in target samples of European ancestries, with markedly worse performance in other

populations, especially in individuals of African descent (Duncan et al., 2018; Martin et al., 2019).

A practical question is how to construct polygenic scores for recently admixed individuals or individuals who are genetically distant from those in the largest existing GWAS. Use of trans-ancestry meta-analytic results to weight alleles can increase prediction accuracy (Grinde et al., 2019), and MultiPred is an approach that combines PRS based on European training data with PRS based on training data from the target population (Márquez-Luna et al., 2017). Current methods development is focused on improving handling of allele frequency differences and LD within and across populations. Given current limitations in understanding similarities and differences in polygenic risk across populations, caution is advised in interpreting differences in PRS across ancestries (Novembre and Barton, 2018).

Heritability and Genetic Correlation

GWAS can provide insights into the genetic architecture of human traits, including **SNP heritability** and **genetic correlation**. Several methods have been proposed for estimating these parameters from genotype data (Supplemental Table S4; Supplemental Methods Section V), but estimation and interpretation of these quantities is more challenging in diverse populations. Heritability estimates may differ between populations due to variation in both environmental factors and population genetic forces. Cross-population differences in phenotype measurement (*Section XI*) may further complicate interpretation. In evaluating shared genetic variance across populations, genetic correlation between groups can be defined either as the correlation of allelic effect sizes (genetic-effect correlation) or the correlation of the relative contribution to total phenotypic variance (genetic-impact correlation), and for all variants or for common variants present in a study. Each value is potentially informative, but divergence in allele frequencies and LD patterns between populations will lead to differences between these parameters (Galinsky et al., 2019).

As detailed in the supplement, most common methods for estimating SNP heritability and genetic correlation either require modification or may not be suitable for use in multi-ancestry studies. Methods relying on relatedness estimation (e.g., genomic relatedness matrix restricted maximum likelihood; GREML) require estimation methods robust to population structure (Conomos et al., 2018; Thornton et al., 2012), and methods modelling LD (e.g., LD Score regression; LDSC) require either ancestry-matched reference panels or individual level data for LD calculations (Luo et al., 2018). Ancestry-matched reference panels, along with the large GWAS sample sizes required for robust estimation using these methods, may be especially challenging to acquire for studies in underrepresented or admixed groups.

Beyond these most common methods, local ancestry tracts in admixed population samples can be leveraged to estimate heritability (Zaitlen et al., 2014) and both genetic-effect and genetic-impact correlations of observed variants can be estimated using Popcorn (Brown et al., 2016) if LD information is available and the two populations are relatively homogeneous. Recent studies estimating cross-ancestry genetic effect correlations have found moderate to high correlations for most phenotypes (Bigdeli et al., 2017; Brick et al.,

2019; Lam et al., 2018). The extent to which these cross-ancestry genetic correlations reflect consistent effects at any particular locus remains a question for fine mapping analyses.

Rare Variant Association Analysis

Rare SNPs and structural variants have been implicated in complex disease (Bomba et al., 2017). Due to their more recent origin, rare variants tend to be more geographically clustered and can be population specific. They can also be particularly important from both clinical and biological perspectives because some confer a large increase in disease risk. However, there is severely limited power to identify trait associations of individual rare variants. Therefore, aggregation methods such as burden tests, variance-component tests, and hybrid tests have been developed to test the combined effect of several variants. Using this approach, variants can be combined within genes or regulatory genetic elements (Gilly et al., 2018; Kuchenbaecker and Appel, 2018). Ancestry groups may carry different driving variants at the same locus, as demonstrated by the association of different functional variants in *ADH1B* with alcohol use disorder in African Americans compared with European and Asian Americans (Edenberg and McClintick, 2018). Therefore, aggregate testing can be particularly suitable to projects involving different ancestral groups because they focus on functional units rather than individual variants and it is not necessary to observe the same variants or frequencies across cases. Meta-analysis methods have been developed that are able to encompass heterogeneous genetic effects across studies and are applicable to cross-ancestry meta-analysis (Lee et al., 2013; Tang and Lin, 2015).

Association testing for rare variants is particularly sensitive to population stratification, and adjusting for fine-scale patterns of population stratification can be difficult with traditional methods (Zhang et al., 2013). In simulation studies, adjusting for PCs failed to fully control inflation for collapsing and variance-component methods (Persyn et al., 2018). Mixed effects models that have been developed for related samples might improve on this (Jiang and McPeck, 2014). However, this area requires further methods development.

Non-Genetic Contributors to Trait Variability

Diversity in social, cultural, and environmental factors also affect disease risk, and can contribute to confounding in genetic studies. In the case of complex traits with strong environmental influences, such as psychiatric conditions, the need to account for non-genetic contributors to disease is important. Unfortunately, measurement of environmental factors can be difficult, so proxy measures such as zip code or insurance status can be used to model non-genetic risk factors such as air quality or accessibility to quality health care. PCs calculated from genotypes can control for population structure due to genetic relatedness, but this approach alone may not capture the social and environmental factors that are encompassed in self-reported “race” and “ethnicity”, even though these measures can be correlated with genetic ancestry. Self-reported measures of diversity can help in the modeling of societal determinants of health, such as increased stress due to the experience of racism and inequality and related variability in environmental factors (e.g., socio-economic status) that affect disease risk. However, the reliance on race and ethnicity as proxy variables for environmental effects or in order to control for population structure may be inappropriate. Better understanding and measurement of causal environmental risk factors is

critical in order to advance discovery methods beyond these over-simplified and potentially harmful constructs of non-genetic contributors to trait variability.

Investigating complex traits in diverse populations, especially when samples are pooled from different research sites or cultural contexts, requires consistency and equivalence in the underlying construct and assessment measures across groups. Differences and variability in **phenotypic measurement** between study sites and populations may affect both gene discovery and the transferability of genetic findings between populations. Most psychiatric classification systems and diagnostic measures have been developed and validated in individuals from industrialized, Western societies (Henrich et al., 2010). This presents a substantial challenge for global and cross-cultural collaborations. Investigations into cross-cultural differences in the prevalence of major depression, for instance, have suggested that although there is a shared underlying disorder construct across groups (Kendler et al., 2015; Simon et al., 2002), individuals may differ culturally in terms of the level of symptomatology reached prior to seeking help (Simon et al., 2002). The inclusion and consideration of diverse populations in the development, validation, and deployment of diagnostic measures used in genetic studies is therefore critical for ensuring an unbiased picture of disease etiology (Supplemental Methods VII).

Despite known large effects of environmental exposures on complex disease risk, there have been limited efforts to incorporate these factors into large-scale genetic studies. Appropriate modeling of the environment is especially critical when a phenotype or trait of interest is influenced by **gene-by-environment interactions (GxE)**. That is, genetic risk factors not only alter average risk but also influence sensitivity to the effects of environmental adversities. However, the majority of GxE studies have been underpowered and conducted using samples of primarily European descent, which limits the assessment of GxE and thereby the identification of modifiable targets for intervention and prevention among understudied groups (Duncan et al., 2014). We note that the statistical definition of GxE depends on the choice of modelling on an additive or multiplicative scale (Kendler and Gardner, 2010). Greater representation of diverse individuals is critically needed in order to increase our understanding of how the interrelated contributions of genes and environment vary across social and cultural groups, and how these factors may interact.

Perspectives and Recommendations

The lack of diversity in genetic studies is problematic for a variety of ethical and scientific reasons. Continued reliance on samples that only represent a fraction of genomic, socio-cultural, and environmental diversity limits our understanding of disease biology and may ultimately contribute to widening global health disparities. Greater ancestral diversity in study samples has the potential to accelerate the discovery of causal risk variants and is critical for a greater understanding of the biological causes of disease, including gene-by-environment interactions. In this Primer, we have highlighted the challenges and benefits of working with diverse populations, recommended practices based on current methods, and have noted specific areas that are in need of further methodological development (Box 3). In summarizing progress, remaining challenges, and requisite next steps, we consider three main domains: 1) researcher participation, 2) data resources, and 3) analytic methods.

Researcher Participation

It is essential that cross-population research is carried out with careful consideration of its ethical, legal, and social implications (ELSI). This includes an ethos of trust-building, transparency, bi-directional knowledge sharing, and community engagement. This is especially true in low and middle income (LMIC) settings and in work with minority groups – contexts in which mistrust of researchers is warranted given historical mistreatment and ethical violations. As there is no single overarching legislative framework that covers this area, we draw attention to literature that (i) articulates key issues (e.g., consent-taking, data-sharing, sample governance, equal partnership, capacity building, community engagement, participants' advisory boards (Akinhanmi et al., 2018; Claw et al., 2018; Parker and Kwiatkowski, 2016) and (ii) proposes effective working solutions to them (Beaton et al., 2017; Campbell et al., 2017; de Vries et al., 2015). Additionally, there is a need to overcome traditional barriers to research empowerment for under-represented groups. H3ABioNet (<https://www.h3abionet.org/>), GINGER (<https://ginger.sph.harvard.edu/>), AMARI (<https://amari-africa.org/>), MIND (<https://minds-uf.org/>), and BRAIN (<https://advance.washington.edu/brains>) are examples of initiatives that embed the targeted delivery of skills and training within broader programs of research. Additional funding mechanisms that support such an approach would be particularly beneficial.

Data Resources

There is a critical need for extensive collaborative efforts to generate large-scale discovery cohorts of diverse ancestry. Limited diversity in genetics research is a major factor limiting our ability to address important scientific questions. The 1KGP (Sudmant et al., 2015) serves as one of the most widely-used resources in genetics research, but expanding those reference panels is a priority. Here, we provide a selected catalogue of extant and emerging sources of whole-genome sequence data (Table 1 and Supplemental Table S3), to facilitate improved matching of diverse study cohorts to appropriate reference panels. Notably, some sources of non-European data are under-utilized, such as minority groups within the UK Biobank. Although diverse ancestry groups only account for about 5% of this data, that fraction amounts to over 35,000 samples of non-European and admixed ancestry (Bycroft et al., 2018) and yet only 7.3% of publications since 2008 that used this data included any of these diverse samples. Thus, there are opportunities to make better use of these and other existing resources.

Additionally, substantial efforts are needed for efficient and ethical international sample and data sharing. This is an issue under active debate, as countries have different approaches to weighing concerns about the privacy of individuals against the collective benefits of science, and the regulatory landscape of individual-level genotype data has been uneven. For example, while the UK allows open access of individual-level genotype data with a valid scientific proposal, other countries, such as Denmark, Iceland, and China, tightly regulate the sharing of such data. Some GWAS consortia, including the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) and Social Science Genetic Association Consortium (SSGAC), overcame these regulatory challenges using essentially a “federated sharing model” (Fiume et al., 2019). Without sharing individual-level genotype data, a study in these consortia follows the prespecified analytic protocol and contributes its summary

statistics to the meta-analysis, allowing the participation of studies that do not have permission to share individual-level data. Researchers should be aware of such options and restrictions, and we recommend regular review of policies as scientific advances may change the ground on which they are based. The practice of sharing summary statistics is increasingly important, and facilitates meta-analyses and other secondary analyses like polygenic risk scoring and estimation of cross-trait genetic correlations. Journals and funding agencies should require sharing of summary statistics whenever it is ethically and legally possible.

Future directions for improving analytic methods

Many of the analytic challenges involved in genetic studies of diverse populations (Box 3) can be addressed by recent advances in methodologies. We reflect on two key issues that remain unresolved and are likely to be beneficial directions for methodological development: 1) the division of individuals into major population groups for analysis and 2) the extension of common secondary analyses of GWAS results to accommodate results from cross-population studies.

A primary question currently faced in genetic analyses of diverse cohorts is whether to follow a ‘combining’ approach (analyzing all individuals together, regardless of ancestry) or a ‘stratifying’ approach (dividing the cohort into major population groups for separate analysis, followed by cross-ancestry meta-analysis; Figure 2). Concerns regarding joint analysis methods (e.g., mixed models) include inadequate control for confounding population stratification and the limited options for secondary analyses such as polygenic risk scoring and genetic correlation estimates. To the extent that stratifying individuals into major population groups remains a feature of cross-population analyses, future methods and theoretical work may continue to refine standards for how best to assign individuals to more homogenous groups. The best solution currently available combines *a priori* analysis plans, exploratory examination of the data, and involving collaborators with expertise in analyzing globally representative datasets. Future work will benefit from increasing diversity in reference panels, formalizing how major populations should be defined for the purposes of genetic analyses, and evaluating the performance of such methods. Continued methodological work should help resolve the tension between these approaches, clarifying if and when stratifying samples is necessary and providing improved methods for joint analysis of diverse cohorts that addresses population stratification.

Many post-GWAS statistical methods have limited portability to association results from diverse and admixed populations, due to complexities with LD patterns. Caution should be taken in the downstream analysis of cross-population GWAS meta-analyses, as many common approaches such as gene-based testing (e.g., MAGMA (de Leeuw et al., 2015)), heritability and genetic correlation estimation (e.g., LD Score regression (Bulik-Sullivan et al., 2015)), and predicted gene expression (e.g., S-PrediXcan (Barbeira et al., 2018)) rely on external reference panels that may not be compatible with the ‘combining’ approach. Even methodologies such as Popcorn (Brown et al., 2016) that are specifically designed for cross-population analyses typically assume single-population summary statistics as input. Furthermore, it is unclear whether annotations of GWAS results based on observed

associations in external studies (e.g., gene expression, Hi-C contacts, methylation) may also need to evaluate population specificity or include diverse samples to improve generalizability across populations. For example, 85% of GTEx eQTL annotations are from individuals of European ancestry (GTEx Consortium, 2013) and other functional genomics resources may be similarly limited.

The above-described methods of cross-population aggregation and comparison rely on an assumption that complex diseases are phenotypically similar across global populations and that measurement of such disorders is culturally unbiased. Given that we know these assumptions are not always accurate, the best practical steps are to be aware of potential phenotypic and environmental differences across populations and involve multi-disciplinary teams with expertise in global societal determinants of health and cultural competency. Suitable methods – such as those that account for cultural context of phenotype ascertainment and GxE – should then be developed and implemented to more precisely measure and treat disorders across cultures.

Conclusion

There is a growing need for investment in policies and practices to support the inclusion of diverse research participants and thus maximize the global potential of genetics research and precision medicine. Broadening participation of both study populations and researchers from many regions of the globe and LMIC in particular will likely be tremendously beneficial. Within the arenas of available data and analytic methods, short-term goals include improved sharing and openness of data. Longer-term goals include identifying ways in which the complex practical, cultural, social, legal and ethical issues inhibiting sample collection from under-represented populations are best resolved. Early, often, and meaningful engagement of stakeholders from diverse patient groups and communities, multi-disciplinary investigators including those with expertise in community-based participatory research, research institutions, scientific editors and reviewers, and funding agencies will all be critical to the success of these short- and long-term objectives towards fostering an environment of inclusive research. Knowing that the lack of representation of diverse populations in genetics research will hinder our understanding of disease etiology, it is clear that this is both an important ethical and scientific growth area for genomics research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1:

Race, Ethnicity, and Ancestry: Interpretation and Relevance for Genetic Diversity.

<p>'Race', 'ethnicity' and 'ancestry' are often used interchangeably, yet they have no universal definitions. We provide brief descriptions of our usage below. For extensive discussion in the context of genomics, including recommendations from professional organizations see: (Banda et al., 2015; Mersha and Abebe, 2015; Race, Ethnicity, and Genetics Working Group, 2005).</p>	
Race	<p>A culturally and politically charged term, for which definitions and meaning are context-specific. Race is related to individual and/or group identity, and is often linked to stereotypes of visible physical attributes such as skin and hair pigmentation. The concept of 'race' is tightly linked to social power dynamics and has historically been used to justify hierarchies of power, discrimination, and oppression in an unequal society. Social and cultural conditions may differ among racial groups, on average, and these differences may lead to environmental effects such as chronic stress and unequal access to goods and services including healthcare and nutrition. These inequities can affect environmental risk for complex diseases and/or potentially interact with genetics to affect risk.</p>
Ethnicity	<p>Describes people as belonging to cultural groups, usually on the basis of shared language, traditions, foods, etc. Ethnicity has often been used interchangeably with 'race,' and is similarly ambiguous. To the extent that traits are affected by social and environmental differences, 'ethnicity' has previously served as a proxy for health and disease risk at the population level as a result of social, cultural, and community effects described above. There is no universal agreement on a system of 'ethnic' groupings worldwide. Some 'ethnic' groups may share genetic factors due to similar ancestral origins, other groups may be more social and cultural in nature.</p>
Ancestry	<p>Meaning varies by context. Here we use the term to denote <i>genetic</i> ancestry, a description of the population(s) from which an individual's recent biological ancestors originated, as reflected in the DNA inherited from those ancestors. Genetic ancestry can be estimated via comparison of participants' genotypes to global reference populations, so incomplete availability of these references can create biased estimates. We note that different methods of calculating genetic ancestry can yield different results. Thus, discrete labelling of ancestral populations over-simplifies the complexity of human genetic variation and demography. Nevertheless, accounting for systematic differences in allele frequencies and LD is necessary for genetic analyses. In this paper, diversity in genomics is described primarily in terms of 'ancestry'.</p>

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Box 2:**General Terminology.**

Term	Definition/Comment
Admixed Population	A population of individuals with ancestors from two or more populations. Admixed can also be used to refer to individuals.
Fine-mapping	Analytical procedures designed to refine GWAS loci to a smaller set of likely causal variant candidates to facilitate interpretation and follow-up studies.
Genetic Correlation	The correlation of genome-wide genetic effects between two phenotypes, which is often estimated for a subset of genomic variants (e.g. SNPs in a GWAS).
Genotype Imputation	Estimation of genotypes at genetic sites that have not been directly measured, using data from a reference panel to infer genotypes based on LD and haplotype structures. Accuracy depends on availability of suitable reference panels.
GWAS	Genome-wide association study. Analysis of common genetic variants across the whole genome for association with a phenotype.
GxE	Gene by environment interaction refers to genetic effects on a phenotype that vary based on environment, or vice-versa.
Haplotype	A group of alleles that are correlated with one another because they are inherited together on a chromosome.
HWE	Hardy-Weinberg equilibrium, the expected balance of genotypes within a population assuming random mating, infinite population size, and no mutation, migration, or selection. Tests of deviations from HWE are used in quality control to detect technical issues with genotyping. Note that there are also non-technical reasons for deviation from HWE (e.g., selection, population structure, admixture, nonrandom mating).
LD	Linkage disequilibrium. Alleles in LD are physically linked on a chromosome, which leads to non-random coinheritance such that their frequencies in a population are correlated.
Major Population	A group of individuals with shared genetic ancestry. A heuristic simplification of the complexity of human demography, but useful for describing groups that are likely to have relatively similar allele frequencies and LD patterns due to shared ancestry. Common examples used in practice include continental ancestry groups or “super populations” as defined by the 1000 Genomes Project (e.g., African, Admixed American, East Asian).
PCA	Principal component analysis. PCA of genotype data is commonly used to examine population structure in a cohort by determining the average genome-wide genetic similarities of individual samples. Derived PCs can be used to group individuals with shared genetic ancestry, identify outliers, and as covariates to reduce false positives due to population stratification.
Population Stratification	Underlying population structure within a sample that is correlated with a phenotype, which can confound genetic association tests.
PRS	Polygenic risk score. A value computed from an individual’s genotype data that quantifies genetic influences on a particular phenotype; also known as polygenic score (PGS), genetic risk score (GRS), or risk profile score (RPS).
Reference Panel	A set of genetic variants from a population. Reference panels are used to design arrays, impute genotypes, catalogue genetic variants, and identify regions that are similar and different between populations.
SNP Heritability	Proportion of phenotypic variance that is explained by additive genetic effects of a set of SNPs.

Box 3:**Common pitfalls, recommendations, and methods in need of development.**

Method	Pitfall	Recommendation	Needs
Genotyping	Many genotyping platforms do not cover non-European variation well.	Use or design population-specific array or multi-ancestry array; high array density can improve coverage in groups with high diversity	Continue improving coverage of diverse ancestries on genotyping arrays
		Consider low-depth whole-genome sequencing	Encourage ongoing development and sharing of pipelines for analysis of low-depth sequencing data
QC	Unnecessary loss of data and/or incorrect inferences by using a one-size-fits-all approach	See Figure 2 for specific recommendations for each QC step and Supplemental Table 2	Improve availability and convenience of implementing proposed QC methods robust to population structure
Imputation	Inaccurate imputation due to poor matching of reference panel to sample	Consider matching the ancestry of the reference panel as closely as possible to the sample ancestry if using a single ancestry sample. Consider the largest reference panel possible for imputation of multiple or admixed samples	Continue expanding diversity of imputation panels, through collection of whole-genome sequencing data, creation of imputation panels from that data, and promoting public sharing/ accessibility of those panels
GWAS	Poor control of population stratification	Consider standard linear/ logistic regression methods for analysis of single ancestry groups followed by meta-analysis. Consider mixed model approaches for admixed or multi-ancestry analyses	Continue investigating causes of - and solutions to - current incomplete control of population stratification from principal components and mixed models
		Include PCs as covariates even when single ancestry groups analyzed. PCs should be computed individually for each major population group within a multi-ancestry cohort and included as covariates in the regression model. Additional covariates should be considered for the multi-ancestry analysis	
Meta-analysis	False negative and false positive findings, effect heterogeneity	Use a random-effects (with possible bias towards the null), or modified random-effects meta-analysis model	Continue to investigate and find solutions to improve power for the detection of heterogeneous effects
Fine-mapping	LD improperly handled when all samples are meta-analyzed across populations	Use fine-mapping methods that explicitly model population-specific LD	Continue to develop fine-mapping methods that rely on fewer assumptions, and thoroughly evaluate their performance
	Uneven genome coverage across populations because of the genotyping array and the imputation reference panel	See recommendations for <i>Genotyping</i> and <i>Imputation</i> above	

Method	Pitfall	Recommendation	Needs
Polygenic risk scores	Loss of accuracy in target population with increasing genetic distance from discovery cohort	Extrapolation of PRS from one ancestry to another is problematic with current approaches and data	Large discovery cohorts for all populations are needed. Develop methods for computing PRS that are not biased when applied across populations, potentially incorporating LD information and/or local ancestry information among diverse populations
Rare variants	Population stratification; low power to detect associations	Aggregate tests can improve power and handle separate causal variants in different populations	Approaches with better control of population stratification; more data on diverse populations needed
Heritability estimates	Differences in MAF and LD structures	For GREML, use admixture-aware relatedness estimation for admixed samples For LDSC, consider using cov-LDSC if in-sample genotype data is available Caution when comparing estimates between groups	Currently no method based entirely on summary statistics can handle admixed/diverse samples. Evaluate options for developing estimation methods with reduced requirements for access to genotype data or ancestry-matched LD reference panels
Cross-ancestral genetic correlation	Different environments Requires large sample sizes and dense array; estimates influenced by genetic distance between groups	Use Popcorn or GREML with admixture-aware estimation of genetic relatedness	Improve robustness and user-friendliness of software for summary statistics; increase diversity of LD reference panels
Phenotypic measurement	Lack of consideration of potential measurement differences across groups	Consider and test for equivalence across populations. Be cautious when meta-analyzing or comparing across groups in which culturally sensitive measurement has not been demonstrated.	Interdisciplinary collaborations with local researchers across populations to continue developing and validating phenotypic measures
GxE	Lack of consideration of environmental factors that are relevant	Consider environmental factors that may be of particular relevance to different socio-cultural groups (e.g., "racial/ethnic" discrimination). Consider running analyses separately for each group to gain understanding of GxE processes within populations, and be cautious when making comparisons across populations	Large samples of diverse individuals and assessment of a broad range of environmental exposures and socio-cultural experiences

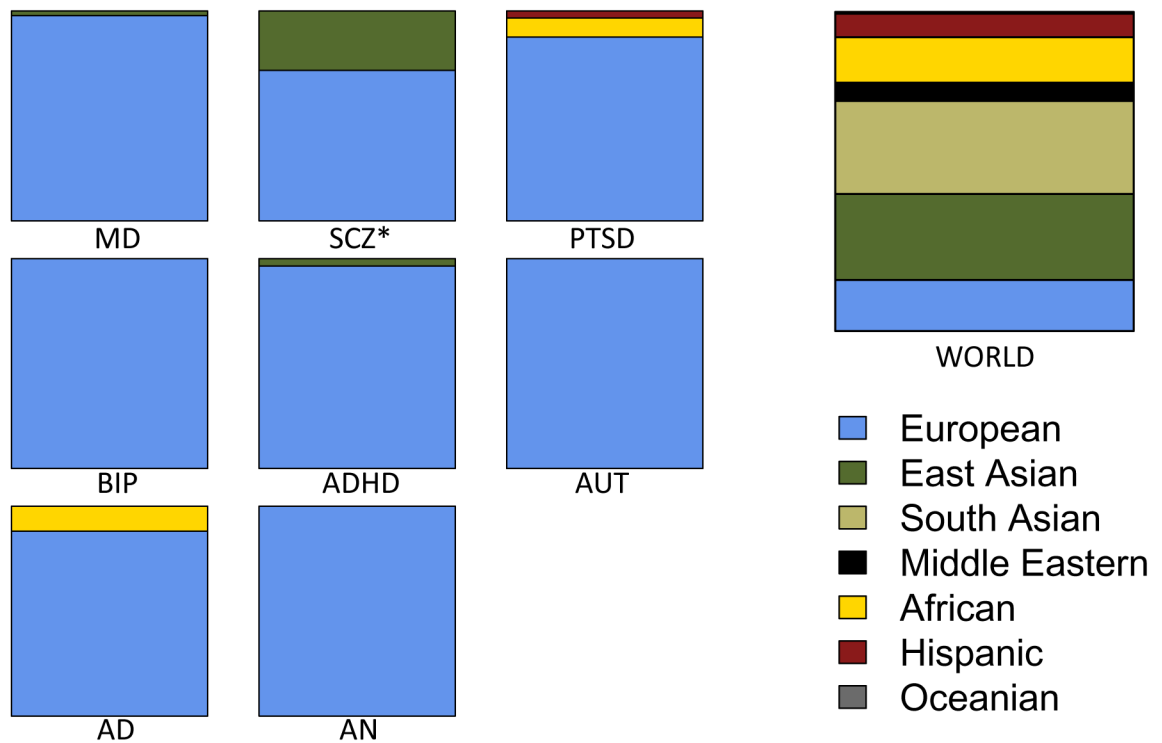


Figure 1. Diversity in GWAS of psychiatric disorders compared to global diversity.

Participant numbers were extracted from the largest consortium publication(s) for each psychiatric disorder and are shown as fractions of the total sample size for each disorder. Note: Sample sizes are given in parentheses. Numbers reflect cases and controls combined. MD=major depression (490,999), SCZ=schizophrenia (205,661), PTSD=post-traumatic stress disorder (188,932), BIP=bipolar disorder (51,710), ADHD=attention deficit hyperactivity disorder (55,230), AUT=autism (46,350), AD=alcohol dependence (52,848), AN=anorexia (14,477). *For schizophrenia, the African American samples from an earlier publication (2009, International Schizophrenia Consortium) were not included in the most recent PGC schizophrenia publication (2014). Ancestry information for each participant was based on principal components analysis of genetic data. See Supplemental Table S1 for consortium studies and references.

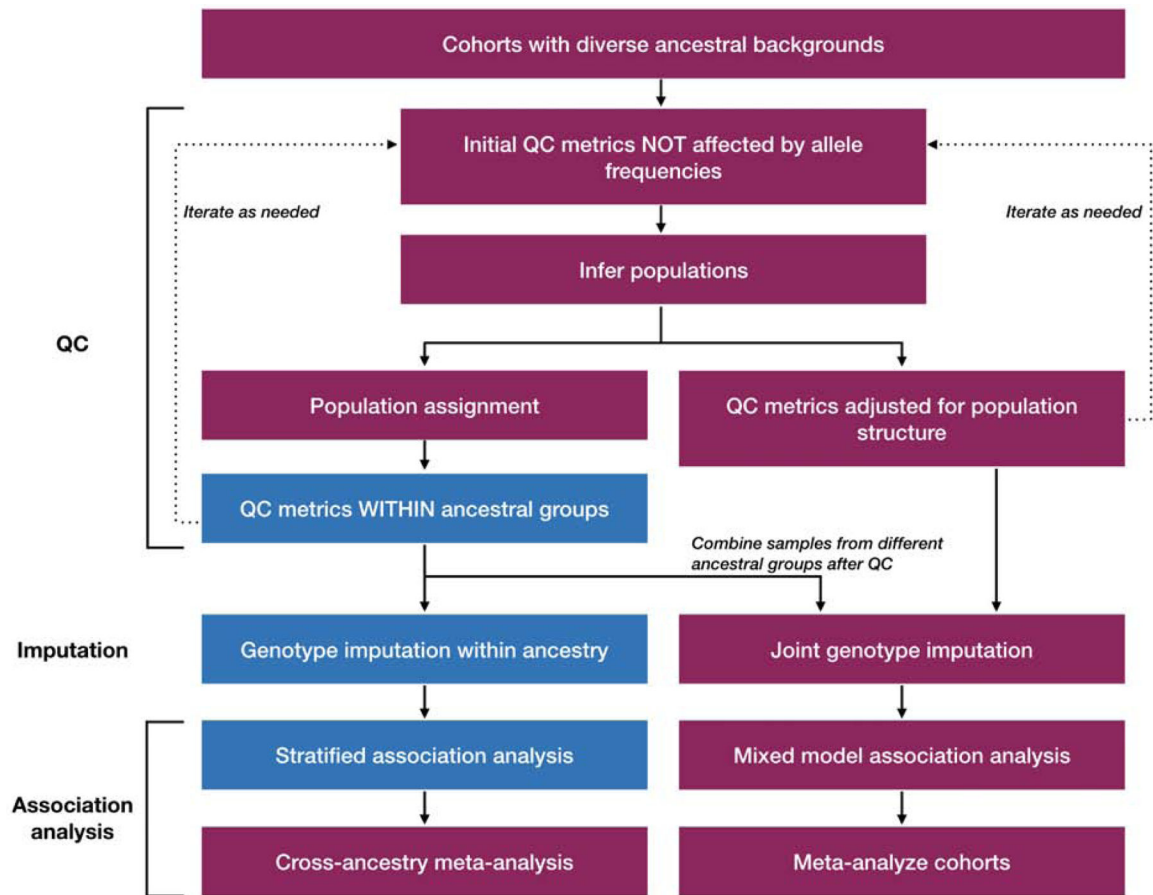


Figure 2: Flow chart for quality control, imputation, and association analysis in diverse population samples.

This flowchart depicts the general analysis framework for genome-wide association studies of participants with diverse ancestral backgrounds. Note: boxes with *red* headers indicate analyses done in samples with diverse ancestral backgrounds and *blue* denotes analysis done within samples in major population groups. The left path shows a strategy for the *stratified meta-analysis approach* and the right path shows steps for the *joint mixed model approach* [see Supplemental Table 2 for more detailed quality control (QC) considerations].

Table 1:

Listing of currently available imputation reference panels.

Reference Panels	Haplotypes	Ancestries	Sites	Availability
TOPMed	125,568	African 32%, Asian 10%, European 40%, Hispanic 16%	463,000,000	forthcoming
Haplotype Reference Consortium (HRC; Version 1.1 2016)	64,940	predominantly European	39,635,008	*, **
African Genome Resources	9,912	African populations + 1000 Genomes Project	93,421,145	**
UK10K	7,562	British population	24,128,798	**
1000 Genomes Project Phase 3 (version 5)	5,008	African 26%, Admixed American 14%, East Asian 20%, European 20%, South Asian 20%	85,167,453	*, **, mathgen.stats.ox.ac.uk/impute
Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA)	1766	Admixed African populations	31,163,897 (autosomes only)	*
Genome of the Netherlands (GoNL)	998	Dutch population	~20,000,000	nlgenome.nl

Note:

* available via Michigan imputation server (<https://imputationserver.sph.umich.edu>).** available via Sanger imputation server (<https://imputation.sanger.ac.uk>). A listing of ongoing projects for imputation panels can be found in Supplemental Table S3.