Article

Genetic Similarity Assessment of Twin-Family Populations by Custom-Designed Genotyping Array

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Abstract

Twin registries often take part in large collaborative projects and are major contributors to genome-wide association (GWA) meta-analysis studies. In this article, we describe genotyping of twin-family populations from Australia, the Midwestern USA (Avera Twin Register), the Netherlands (Netherlands Twin Register), as well as a sample of mothers of twins from Nigeria to assess the extent, if any, of genetic differences between them. Genotyping in all cohorts was done using a custom-designed Illumina Global Screening Array (GSA), optimized to improve imputation quality for population-specific GWA studies. We investigated the degree of genetic similarity between the populations using several measures of population variation with genotype data generated from the GSA. Visualization of principal component analysis (PCA) revealed that the Australian, Dutch and Midwestern American populations exhibit negligible interpopulation stratification when compared to each other, to a reference European population and to globally distant populations. Estimations of fixation indices ($F_{ST}$ values) between the Australian, Midwestern American and Netherlands populations suggest minimal genetic differentiation compared to the estimates between each population and a genetically distinct cohort (i.e., samples from Nigeria genotyped on GSA). Thus, results from this study demonstrate that genotype data from the Australian, Dutch and Midwestern American twin-family populations can be reasonably combined for joint-genetic analysis.

Keywords: Genetic similarity assessment; genotyping microarray; population genetics; population structure; principal component analysis; twin

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Scientific investigations aimed at disentangling the contribution of genetic factors underlying complex and polygenic traits have demonstrated the necessity of large sample sizes (Evangelou et al., 2018; Wray et al., 2018). Only when sample sizes are vast is it possible to estimate the contribution of each locus influencing a complex trait (Tam et al., 2019; Vischer et al., 2012, 2017). It is both difficult and financially challenging for a single site to accrue large enough sample sizes to achieve adequate statistical power. Therefore, one pragmatic approach for obtaining the large number of samples required is to aggregate samples collected by different groups, either through meta- or mega-analysis. Currently, twin registers from around the world routinely employ this strategy for genotypic and phenotypic data (Silventoinen et al., 2015, 2016). This approach is powerful if genetic heterogeneity (e.g., as a result of dissimilar population ancestry and demographic histories) is not an issue or is appropriately accounted for. Here, we explore the degree of genetic similarity between multiple twin cohorts and indicate whether it is appropriate to combine data from these cohorts for joint-genetic analysis.

In 2006, a study by Sullivan et al. (2006) empirically showed that samples from Australian and Netherlands Twin Registers can be reasonably combined for joint-genetic analyses by estimating the proportion of total genetic variability attributable to genetic difference between cohorts (Sullivan et al., 2006). The calculation of the genetic variability attributable to genetic differences between cohorts, measured by Wright’s fixation index ($F_{ST}$ value), was estimated using analysis of molecular variance on 359 short tandem repeat polymorphism markers. The estimated $F_{ST}$ between Australian ($N = 519$) and the Netherlands ($N = 549$) was found to be 0.30%, a value smaller than between many other European groups. The $F_{ST}$ estimates suggested that it is reasonable to combine samples from Australian and Dutch cohorts but admittedly based on calculations in samples of modest size. Here, we evaluate the genetic similarity in larger number of samples and augment the comparison by adding a third cohort of samples obtained from the Avera Twin Register (ATR), a representative population sampling of the Midwestern region of the USA. In this study, we test the genetic variation within and between three populations of interest — Australian, Dutch and Midwestern American — by employing genomic data from a custom-designed genome-wide single nucleotide polymorphism (SNP) array. To further explore the genetic similarity across the cohorts under study, we incorporated genetic data from a globally and genetically distinct population — samples
Table 1. Characteristics of samples genotyped on GSA per cohort and tissue

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Country of origin</th>
<th>N</th>
<th>Female (%)</th>
<th>Composition</th>
<th>Unrelated individuals</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avera Twin Register</td>
<td>USA</td>
<td>602</td>
<td>66.4</td>
<td>MZ, DZ, parents, sibs</td>
<td>238</td>
<td>Buccal</td>
</tr>
<tr>
<td>NTR</td>
<td>Netherlands</td>
<td>1135</td>
<td>55.4</td>
<td>MZ, DZ, parents, sibs</td>
<td>6139</td>
<td>Blood</td>
</tr>
<tr>
<td>NTR</td>
<td>Netherlands</td>
<td>9091</td>
<td></td>
<td>MZ, DZ, parents, sibs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australian Twin Register</td>
<td>Australia</td>
<td>1922</td>
<td>100</td>
<td>MODZT</td>
<td>1448</td>
<td>Blood</td>
</tr>
<tr>
<td>Nigerian Twin Register</td>
<td>Nigeria</td>
<td>100</td>
<td>100</td>
<td>MOSDZ</td>
<td>96</td>
<td>Saliva</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12,850</td>
<td></td>
<td></td>
<td>7921</td>
<td></td>
</tr>
</tbody>
</table>

GSA = Global Screening Array; NTR = Netherlands’s Twin Register; N = number of samples; MZ = monozygotic twins; DZ = dizygotic twins; MODZ = mothers of DZ twins; MOSDZ = mothers of opposite-sex dizygotic twins; sibs = siblings; parents = parents of twins.

Percentage of female samples is reported from the unrelated set.

Materials and Methods

Participants and Sample Collection

Australian subjects were from the QIMR Berghofer Medical Research Institute (N = 1922; Hopper, 2002, 2006). Other subjects were registered participants of the Netherlands (NTR, N = 10,226; Boomsma et al., 2006) and Avera (ATR, Sioux Falls, SD, USA, N = 602; Kittelsrud et al., 2017) Twin Registers, and the Nigerian Twin and Sibling Registry (NTSR, N = 100; Hur et al., 2013; see Table 1).

Representative samples of the Midwestern American population were obtained from the ATR. Enrolled participants include twins, multiples, siblings and their parents. Participants complete surveys and questionnaires and provide a cheek swab (buccal brushing) for zygozity testing and genotyping. The majority of the enrolled participants are located in the Midwestern region of the USA, with most being from South Dakota, Minnesota and Iowa.

Samples from the QIMR Berghofer Medical Research Institute (Australia) are a combination of a number of different studies, conducted in many countries over many decades, focused on the genetics of dizygotic twinning (Painter et al., 2010). Samples from
mothers of dizygotic twins (MODZT) were collected from Australia and New Zealand and were shipped to the AIHG for genotyping on the GSA regardless of whether they were ungenotyped or previously genotyped on an earlier SNP array. Included in the shipment were two small cohorts of special interest: (1) a Belgian sample of 40 MODZT from 14 multiplex families collected in the 1990s; (2) a sample of 10 MODZT from two multiplex families from the Utah Mormon Database collected in 1994. For the purposes of the study presented here, samples from Belgium and Utah were excluded from the Australian cohort.

The Nigerian sample in the present study was drawn from the NTSR, which included over 3000 adolescent monozygotic and dizygotic twins, their parents and siblings, collected mainly from public schools in Lagos State and Abuja, Federal Capital Territory in Nigeria. Participants of the NTSR completed questionnaires and provided saliva or buccal samples for genotyping. The sample used in the present study consisted of 100 mothers of opposite-sex twins attending public schools in Lagos State, collected for the purpose of a pilot study to understand the genetic underpinnings of dizygotic twinning. Lagos State is located in the southwestern geopolitical zone of Nigeria and one of the most populous urban areas in Nigeria. Although residents of Lagos State are ethnically diverse, they are mainly members of the Yoruba group.

Participants from the NTR included twins, their parents and other relatives (mainly siblings of twins). NTR participants take part in survey and other research projects and provided blood or buccal samples for DNA isolation and genotyping.

**DNA Extraction and Genotyping**

DNA was isolated from whole blood, buccal epithelial cells (Min et al., 2006) and saliva using standard protocols for downstream SNP genotyping. High-density SNP genotyping for all samples was done at the AIHG using a custom-designed Illumina GSA according to the manufacturer’s protocol.

**Design of a Customized Genotyping Array and Generation of Genotype Data**

The GSA employed for this study was designed following a previously defined strategy for designing population-specific customized genotyping arrays (Ehli et al., 2017). Specifically, the GSA was custom designed to contain a core imputation backbone (approximately 660,000 markers) based on commonly utilized reference panels, such as the GoNL (Boomsma et al., 2014) and the 1000 Genomes Project (Genomes Project Consortium et al., 2015). In addition to the core backbone, the array includes approximately 30,000 additional markers for fine mapping to further enhance imputation quality, and 8000 markers of interest associated with a variety of conditions, disorders and traits, including neuropsychiatric disorders, drug metabolism, fertility and twinning (Table 2). In total, the GSA contains 697,486 markers.

Prior to design of the array, initial validation of the GSA content for imputation was assessed by checking concordance, coverage and imputation quality using an extracted subset of markers resembling the GSA (683,937 markers). In brief, a dataset mimicking the content on the GSA was curated from 249 unrelated female individuals of the GoNL project. Males from the GoNL were excluded as the tools used for assessment of genotyping array coverage could not properly handle a homozygous X chromosome. GSA-mimicked markers were quality controlled and retained if minor allele frequency (MAF) was >0.01, missingness per individual was <10%, missingness per SNP was <5% and if there was no statistically significant deviation from Hardy–Weinberg equilibrium ($p > 10^{-5}$). Quality control and filtering reduced the number of markers to 617,340. Extracted and quality-controlled markers were then selected if they were present in the 1000 Genomes (1000G) reference panel (Genomes Project Consortium et al., 2015; 616,961 markers). The extracted set was phased with SHAPEIT (Delaneau et al., 2011) and imputed against the 1000G reference panel phase 3 using IMPUTE2 (Howie et al., 2009). For the ~12.1 million overlapping markers, concordance was calculated in PLINK (Chang et al., 2015; Purcell et al., 2007) by comparing the 1000G best-guess genotypes to the original GoNL genotypes.

<table>
<thead>
<tr>
<th>Marker type</th>
<th>Number of SNPs (N = 697,486)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSA core backbone</td>
<td>Total ~660,000</td>
</tr>
<tr>
<td>Sex chromosomes</td>
<td>17,880 X; 1480 Y; 578 PAR</td>
</tr>
<tr>
<td>ADME genes/exons</td>
<td>6668; 2787</td>
</tr>
<tr>
<td>ClinVar</td>
<td>17,020</td>
</tr>
<tr>
<td>MHC</td>
<td>9797</td>
</tr>
<tr>
<td>Ancestry informative</td>
<td>3212</td>
</tr>
<tr>
<td>Fine-mapping content (candidate genes and additional markers for imputation)</td>
<td>Total ~30,000</td>
</tr>
<tr>
<td>Custom markers</td>
<td>Total ~8000</td>
</tr>
<tr>
<td>Fertility and twinning</td>
<td></td>
</tr>
<tr>
<td>Body stature (height, BMI) and sports and exercise behavior</td>
<td></td>
</tr>
<tr>
<td>Mental state and health (happiness, depression, schizophrenia)</td>
<td></td>
</tr>
<tr>
<td>Chromosome X (imputation)</td>
<td></td>
</tr>
<tr>
<td>Educational attainment</td>
<td></td>
</tr>
<tr>
<td>Pharmacogenomics</td>
<td></td>
</tr>
</tbody>
</table>

GSA = Global Screening Array; ADME = absorption, distribution, metabolism, excretion; BMI = body mass index; ClinVar = NCBI archive for interpretations of clinical significance of genetic variants; MHC = major histocompatibility complex; PAR = pseudoautosomal region.

Table 2. Content and marker selection categories of the custom-designed Illumina GSA
Following initial clustering, cluster positions were evaluated and edited based on sequential assessment of several cluster metrics. Cluster positions were zeroed (resulting in no genotype calls for a locus) based on low cluster separation (≤0.27), low call frequency (≤0.96), low mean normalized intensity values for the heterozygote genotypes (≤0.2), extreme mean normalized theta values of the heterozygote cluster (<0.2 or >0.825), Mendelian inconsistencies, ambiguous clusters, excessive numbers of reproducibility errors and excessive heterozygote calls relative to expectations based on Hardy–Weinberg equilibrium (>0.2). Markers on X and Y chromosomes were manually evaluated and edited on a per-marker basis.

**Data Management, Quality Control and Relationship Inference**

Individual samples were removed if they had a missing rate greater than 10% or excess genome-wide inbreeding levels/heterozygosities (as calculated in PLINK, F coefficient <−0.10 or >0.10). Reported sex was compared with inferred sex from the genotype data. Sex mismatches were investigated, resolved and subsequently replaced in the dataset.

From each population sample, we selected the largest group of unrelated individuals (Table 1). Unrelated individuals were identified with KING software (Manichaikul et al., 2012) using the ‘−unrelated’ option. In brief, related individuals (estimated kinship coefficient <0.088) were clustered into families. Within each connected group, individuals were ranked according to the count of unrelated family members, corresponding to an estimated kinship coefficient <0.022. A set of unrelated individuals was then made by selecting the individuals with the largest count of unrelated individuals within the respective family group. Additional unrelated individuals were obtained by taking the individuals with the next most unrelated family members, only if that individual was not related to any of the previously selected unrelated individuals. The final selection contained no pairs of individuals with a first- or second-degree relationship, reducing the sample size to 7921 subjects. Following quality control, the number of samples was reduced to 7782.

**SNP Quality Control and 1000G Alignment**

All autosomal SNPs that passed quality control and filtering were analyzed. PLINK was used to perform quality control. A selection of highly performing markers (N = 564,020) was used for subsequent quality control and analyses. Specifically, SNPs were removed if they were not in the 1000G reference panel (phase 3 version 5) or if they were palindromic SNPs with an allele frequency of 0.40–0.60. Polymorphic SNPs with more than two alleles were also excluded. SNP marker names were adjusted for congruity of highly performing markers (PLINK. The merged set was filtered to remove markers with a MAF <0.05, pruned for LD and excluded SNPs in long-range LD). Quality control and filtering reduced the final number of autosomal SNPs from 564,020 to 109,702 SNPs. This number of SNPs was used for comparisons between samples genotyped on GSA, namely those from the Australian, Midwestern American, Dutch and Nigerian cohorts.

We also calculated whether there were statistically significant pairwise differences between the Australian, Dutch and Midwestern American populations and representative European populations from the HGDP using smartpca. For each pair of populations, ANOVA statistics along each eigenvector were summed across all 10 eigenvectors.

**HGDP Data Management and Projection**

To establish genetic similarity on a global scale, PCs of the Australian, Dutch, Midwestern American and Nigerian populations were projected onto samples obtained from the HGDP (Cann et al., 2002; Rosenberg, 2006). The HGDP data comprise genotypes (660,918 SNPs) from 1043 fully consenting individuals representing 54 global populations from Sub-Saharan Africa, North Africa, Europe, the Middle East, Central and South Asia, East Asia, Oceania and the Americas and provide a representative sampling of worldwide genetic variation (available at https://www.hgsc.org/hgdp/files.html).

Raw genetic data from the HGDP (sample call rate >98.5%) were reformatted for PLINK using command line tools. Markers with greater than 5% missingness were removed. Following the same procedures as previously described, unrelated individuals were identified in the HGDP dataset and retained using the program KING. Removal of related individuals reduced the sample size from 1043 to 857. To be consistent with GSA, HGDP data were converted from Build 36.1 coordinates to Build 37/hg19 using University of California, Santa Cruz (UCSC’s) batch coordinate conversion tool, liftOver (Haessler et al., 2019; Kent et al., 2002). Overlapping markers between HGDP and GSA (prior to MAF filter, LD pruning and exclusion of long-range LD) were identified in the variant information files (.map) using R (R Core Team, 2018). Of the 133,835 common markers between the datasets, there were 21,667 multi-allelic variants due to strand inconsistencies. Strand flips were resolved and data from the HGDP were merged with cleaned and filtered GSA data using PLINK. The merged set was filtered to remove markers with a MAF <0.05, pruned for LD and excluded SNPs in long-range LD. Quality control and filtering reduced the final number of markers to 54,820.

Ten PCs were calculated using smartpca within EIGENSOFT with default parameters. All HGDP populations were specified as reference populations for the PC projection.

**Case-Control GWA Study**

We performed case-control GWA study (GWAS) between the Midwestern American, Australian and Dutch populations to gain insight into the degree of genetic relatedness between them. To avoid false positives, we excluded variants with a MAF <0.10 in the quality-controlled and filtered data on unrelated individuals. Simple association testing was done in PLINK with the ‘−assoc’ command. Two GWAs were performed, both with the Midwestern American population defined as cases and with
Dutch and Australian samples serving as controls. Manhattan plots and quantile–quantile (QQ) plots were created to visualize regions of the genome that appeared statistically significant.

### Calculation of $F_{ST}$ Estimates

To quantify measures of structure in populations, we estimated $F_{ST}$ values between the Midwest American, Australian, Dutch and Nigerian cohorts. Weir and Cockerham (1984) and Hudson et al. (1992) estimators were calculated using two different software programs: popstats (Skoglund et al., 2015) and scikit-allel (Miles 2018), implemented in Python.

### Results

#### Validation of the GSA

Imputation quality metrics, quantified by $R^2$ values, are presented in Table 3. For all 1000G imputed autosomal SNPs, including those present in African and Asian populations, the median $R^2$ values for the GSA are 0.02 for MAF > 0.000–0.001, 0.69 for MAF > 0.001–0.01, 0.97 for MAF > 0.01–0.05 and 0.99 for MAF > 0.05. For the selection of autosomal SNPs that were present in both the GoNL and 1000G reference data, indicative of true genetic variants in the Dutch population, the results demonstrate improved imputation quality compared to all SNPs present in 1000G. The median $R^2$ values of the SNPs in the GoNL and 1000G reference set are 0.04 for MAF > 0.000–0.001, 0.80 for MAF > 0.001–0.01, 0.97 for MAF > 0.01–0.05 and 0.99 for MAF > 0.05. Here, the improved imputation quality, captured by both median and mean scores, is mainly the result of the exclusion of a large number of rare SNPs (i.e., SNPs in African and Asian populations — captured by the full 1000G set), which are likely absent from the Dutch population.

Concordance of the genotyped GoNL SNPs that were reimputed with a 1000G imputation reference panel was high for most SNPs in the genome, as can be seen in Table 4. In the imputed data, of the 12,074,470 polymorphic variants with a MAF > 0, up to 62.2% can be reimputed with very high quality. At lower levels of quality (below 80% concordant), 1.95% of the genome is not well covered.

### Principal Component Analysis

We performed fine-scale PCA of unrelated subjects from the Australian, Dutch and Midwestern American populations to investigate the degree of genetic relatedness of these populations independent of other global populations. The PCA utilized 109,702 autosomal SNPs after stringent quality control, filtering, pruning and exclusion of long-range LD regions. As seen in Figure 1, results of the PCA suggest that the Midwest American, Australian and Dutch populations are not genetically distinct from one another since the clusters moderately overlap. The Midwest American cluster partially superimposes both Australian and Dutch clusters, which themselves also show a small degree of overlap. Visualization of PCs from the PCA on the Australian, Dutch and Midwestern Americans demonstrates commonality of population clusters, thereby suggesting a high degree of genetic similarity between these populations.

Provided the unique opportunity to genotype Nigerian mothers of twins on the GSA, a PCA was performed with the inclusion of these globally distinct samples to serve as a genetic contrast group to the populations under study. Thus, to enhance the investigation of the genetic similarity of the Australian, Dutch and Midwestern American populations on a broader scale, we performed a PCA on all samples genotyped on GSA at AIHG, including those from Nigeria. The results of the PCA are depicted in Figure 2. Inclusion of a geographically and genetically distant population resulted in distinct separation of European-ancestry-based populations and the Nigerian cohort, indicative of population stratification and genetic dissimilarities.

### Table 3. Imputation quality metrics per minor allele frequency bin for the GSA

<table>
<thead>
<tr>
<th>Selected SNPs</th>
<th>Chr</th>
<th>MAF range</th>
<th>N SNPs</th>
<th>Median $R^2$</th>
<th>Mean $R^2$</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000G All SNPs$^a$</td>
<td>1-22</td>
<td>&gt;0.000-0.001</td>
<td>21,373,838</td>
<td>0.02</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.001-0.01</td>
<td>6,853,643</td>
<td>0.69</td>
<td>0.64</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.01-0.05</td>
<td>2,863,052</td>
<td>0.97</td>
<td>0.91</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.05</td>
<td>6,974,825</td>
<td>0.99</td>
<td>0.96</td>
<td>0.08</td>
</tr>
<tr>
<td>GoNL and 1000G$^b$</td>
<td>1-22</td>
<td>&gt;0.000-0.001</td>
<td>1,003,022</td>
<td>0.04</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.001-0.01</td>
<td>2,736,096</td>
<td>0.80</td>
<td>0.74</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.01-0.05</td>
<td>2,461,024</td>
<td>0.97</td>
<td>0.92</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;0.05</td>
<td>5,874,328</td>
<td>0.99</td>
<td>0.97</td>
<td>0.07</td>
</tr>
</tbody>
</table>

$^a$Denotes full 1000G imputation with Asian/African/other SNPs not present in the Dutch population.

$^b$Denotes overlapping SNPs between GoNL and 1000G. All monomorphic SNPs were excluded, thus only polymorphic SNPs were selected for each comparison.

### Table 4. Genotype concordance metrics for GSA-mimicked, genotyped GoNL SNPs that were reimputed with 1000G reference panel

<table>
<thead>
<tr>
<th>Concordance (%)</th>
<th>N SNPs</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;99</td>
<td>7,506,660</td>
<td>62.17</td>
</tr>
<tr>
<td>95–99</td>
<td>3,470,030</td>
<td>28.74</td>
</tr>
<tr>
<td>80–95</td>
<td>861,745</td>
<td>7.14</td>
</tr>
<tr>
<td>50–80</td>
<td>221,831</td>
<td>1.84</td>
</tr>
<tr>
<td>≤50</td>
<td>14,204</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Note: Total number of 1000G SNPs that were reimputed, polymorphic and present in GoNL = 12,074,470.

GSA = Global Screening Array; GoNL = Genome of the Netherlands; $N$ SNPs = number of SNPs.

References:

- Hudson et al. (1992)
- Skoglund et al., 2015
- Miles 2018
Projection of PCs from all samples genotyped on GSA onto those from the HGDP allowed for visualization of populations on a globally diverse scale. Results of the PCA using the HGDP as reference populations showed clear layering of the Australian, Dutch and Midwestern American populations on the representative European HGDP cohort (Figure 3). The HGDP European population is comprised of samples collected from France, Italy, Italy-Bergamo, Orkney Islands, Russia and Russia-Caucasus. At the global level, the Australian, Dutch and Midwestern American samples showed strong distinction from African and Asian populations. Alternatively, the PCs of the cross-ethnic comparison demonstrated strong distinction from African and Asian populations. Alternatively, the PCs of the cross-ethnic comparison demonstrated strong distinction from African and Asian populations.

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Fig. 1. Genetic ancestry of Midwestern American, Australian and Dutch subjects. Shown are the results from PCA using autosomal genotyped SNPs after quality control, filtering, pruning and exclusion of long-range LD (109,702 markers). Ancestry outliers were removed prior to performing PCA. PC1 and PC2 represent the first and second PCs and account for 18.864% and 11.919% of the variation, respectively.

Fig. 2. Genetic ancestry of Midwestern American, Australian, Dutch and Nigerian subjects. Shown are the results from the PCA using all autosomal genotyped SNPs after quality control, filtering, pruning and exclusion of long-range LD (109,702 markers). Ancestry outliers were removed prior to performing PCA. PC1 and PC2 represent the first and second PCs and account for 67.765% and 6.568% of the variation, respectively.

Table 5. Statistical significance of differences between populations

<table>
<thead>
<tr>
<th>Population 1</th>
<th>Population 2</th>
<th>Chi-square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midwestern American</td>
<td>Netherlands</td>
<td>457.171</td>
<td>6.169 × 10⁻⁹²</td>
</tr>
<tr>
<td>Midwestern American</td>
<td>Australian</td>
<td>660.324</td>
<td>2.053 × 10⁻¹³⁵</td>
</tr>
<tr>
<td>Australian</td>
<td>Netherlands</td>
<td>7121.469</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: For each pair of populations, ANOVA statistics along each eigenvector were summed across eigenvectors. Degrees of freedom is equal to 10, the default number of eigenvectors.

In order to provide a quantitative estimate of relationships between populations in a pairwise fashion, we used smartpca to sum ANOVA statistics across all eigenvectors (parameter default of 10 eigenvectors). The results of the pairwise comparisons of the Australian, Dutch and Midwestern Americans are shown in Table 5. Statistically significant differences were observed for each pairwise comparison, suggesting the existence of population stratification.

To put in context the genetic differences between the Australian, Dutch and Midwestern Americans, we tested for significant differences between them and the samples obtained from Nigeria, as well as all HGDP populations, including representative European populations (Supplementary Files 1 and 2). All comparisons between the Australian, Dutch, and Midwestern American and HGDP European populations were statistically significant, with the comparison between Australian and Orkney Islands populations being least significant. More generally, for each cohort, the statistical comparison with the Orkney Islands resulted in the least significant difference.

Case-Control GWAS

We performed two case-control GWAS between Midwestern American (cases) and Australian (controls) and Dutch (controls) populations using only common variants. A MAF filter (MAF > 0.10) was employed to avoid false positives due to minor allele frequencies. The GWAS between Midwestern American (227 cases) and Australian (1354 controls) utilized 228,166 variants after MAF filtering. Results of the case-control GWAS between
the two populations are visualized in a Manhattan plot (Figure 4(C)). Four chromosomal regions exhibited genome-wide significant differences ($p < 5 \times 10^{-8}$). The dbSNP ID numbers for the significant SNPs are rs6420020 (chromosome 5, $p = 1.571 \times 10^{-15}$), rs10817415 (chromosome 9, $p = 3.852 \times 10^{-15}$), rs11599284 (chromosome 10, $p = 5.387 \times 10^{-14}$) and rs78611721 (chromosome 20, $p = 2.164 \times 10^{-14}$). The frequency of these SNPs was much greater in Australians than in American individuals. The QQ-plot (Figure 4(D)) of genome-wide $p$ values showed modest deviation from the null hypothesis of no association. The overall GWAS genomic control statistic ($\lambda$) was 1.153, indicating slight inflation due to population structure, driven by a small number of polygenic variants, between the Midwestern American and Dutch populations.

The second case-control GWAS was performed between Midwestern American (227 ‘cases’) and Dutch (6139 ‘controls’) using 228,025 common variants after MAF > 0.10 filtering. The results of the case-control GWAS between the two populations are presented in the Manhattan plot in Figure 4(A). No statistically significant variants exceeded the genome-wide significance threshold ($p < 5 \times 10^{-8}$). The QQ-plot (Figure 4(B)) shows slight genomic inflation across the entire range of $p$ values. The GWAS genomic control statistic ($\lambda$) was 1.159, again indicating slight inflation due to population structure differences between the Midwestern American and Dutch populations.
**FST Estimates**

FST values were calculated as a measure of genetic differentiation between populations. We generated FST values using two approaches, namely Weir and Cockerham (1984) and Hudson et al. (1992) estimators. As demonstrated by Bhatia et al. (2013), the Weir and Cockerham estimator is dependent on the ratio of sample sizes comprising each population. Therefore, an alternative approach is to use the Hudson estimator, which can be implemented as a strategy independent of sample sizes, even when FST is not uniform across populations. The result produced by the Hudson estimator is a simple average of the population-specific estimators originally defined by Weir and Hill (2002). Ultimately, the Hudson estimator was recommended by Bhatia et al. (2013) or estimating FST for pairs of populations with unequal sample sizes.

The FST estimates from both the Weir and Cockerham and Hudson estimators are shown in Table 6 and are relatively close to previously reported estimates from the HapMap consortium (International HapMap 3 Consortium et al., 2010) and GoNL project (see Supplementary Table 5 in Genome of the Netherlands Consortium, 2014). Regardless of the estimator, smaller FST estimates are observed between the Australian, Dutch and Midwestern American populations than between each population compared to the Nigerian cohort. Consistent with the work of Bhatia et al. (2013), it is important to note that the choice of the estimator made an impact on the resulting FST estimate.

**Discussion**

To gain insight into the routine practice of aggregating genomic datasets from twin registers from around the world, we investigated interpopulation genetic variation with genome-wide data generated on GSA from the Australian, Dutch and Midwestern American populations. Here, we report on the inception and initial validation of a custom-designed Illumina GSA and its implementation in studying population genetic variation. Through quantitative measures and visualization of PCs, results of work presented here suggest a high level of genetic similarity between the Australian, Dutch and Midwestern American populations, albeit with small, yet statistically significant differences existing between them.

The custom-designed GSA provides a genotyping platform initially optimized for imputation containing a core imputation backbone supplemented with additional fine-mapping content to bolster imputation quality and genome-wide coverage. Also featured on the GSA are custom-selected markers specific to phenotypes of interest, notably for fertility and twinning.

With the use of the GoNL reference set and a selection or markers mimicking the GSA, we demonstrated that we can re-impute genotypes with a high degree of confidence. Exceptions are made for rare alleles (MAF < 0.0001), which are never well imputed (Zheng et al., 2015). A limitation to the validation of the GSA is that we utilized the sequences of only 249 samples; therefore, the imputation and presence of alleles with a MAF < 0.01 was likely less than ideal. However, by comparing the validation results of the Illumina GSA to other commercially available genotyping platforms the imputation quality of the GSA is well in line with other genotyping products (refer to Tables 1 and 2 in Ehli et al., 2017). Additionally, the method of testing the coverage using two reference datasets to check concordance between genotyped and reimputed genotypes utilizes SNPs in union with both reference panels. Thus, we inherently assume that SNPs specific to a population — for example, those SNPs only appearing in GoNL — are covered and imputed in such a manner.

Nevertheless, the GSA has been instrumental in generating high-quality genotype data from cohorts around the world for use in population genetic studies of complex traits.

PCs often show remarkable correlation with geography, a manifestation of decreasing genetic similarity with increasing geographic distance. Thus, in an effort to elucidate the degree of genetic resemblance between the Australian, Dutch and Midwestern American populations, we performed PCA and visualized PCs. Visualization of PCs for the three populations under study shows a high degree of overlap between PCs 1 and 2. The similarity observed between Midwestern American and Dutch populations is consistent with estimates of 4.1 million Americans (1.28% of the US population in 2017) claiming total or partial Dutch heritage (Data Access and Dissemination Systems, 2017). In large part, the majority of inhabitants of Midwest America have ancestral origins rooted in Northwestern Europe as a result of common migratory routes. Lending additional support to the Midwestern American and Dutch similarity is the fact that the majority of the Dutch Americans reside in Michigan, California, Montana, Minnesota, New York, Wisconsin, Idaho, Utah, Iowa, Ohio, West Virginia and Pennsylvania. Together, it is apparent that there is strong Dutch influence and saturation in the Midwestern region, which is reflected in the genetic profiles of these populations.

Broad-scale comparison to diverse populations from around the world, such as those represented by the HGDP, further portrayed similarity between the Australian, Dutch and Midwestern Americans and with European populations more generally. The close resemblance of Australian and European populations is consistent with prior empirical results (Stankovich et al., 2006) and the fact that immigrants from Northern Europe colonized Australia (mainly from Britain and Ireland) and America. Incorporation of genotype data from a globally distinct population (i.e., Nigerian samples genotyped on GSA) facilitated the projection of the PCs onto the HGDP and recapitulated worldwide genetic diversity.

Quantitative measures of population similarity, as measured by summed ANOVA statistics over eigenvectors, revealed small yet statistically significant differences between the Australian, Dutch and Midwestern American populations. Additional comparisons of each population to individual HGDP European cohorts further demonstrated significant differences suggestive of population stratification. Likewise, patterns of FST estimations were consistent with the geographical clustering observed in PCA and with previous FST estimates of global population genetic differentiation. Altogether, it is likely that the observed population genetic dissimilarities are due to systematic allele frequency differences resulting from migration, adaptation, drift and selection.

In general, large GWAS efforts aimed at discerning the genetic contributions to complex traits typically rely on meta-analyses of multiple cohorts of relatively homogeneous populations. Thus, to assess the level of homogeneity between Midwestern American, Australian and Dutch cohorts, we performed case-control association testing between populations. Case-control GWAS of Midwestern American and Australian populations yielded results to suggest that only small genetic differences exist between the populations under study. We hesitate to interpret the few differences observed between the Australian and Midwestern American populations, although we note that it is possible that the genome-wide significant SNPs (rs6420020, rs10817415, rs78611721 and rs11599284) could be implicated in the dizygotic twinning phenotype given that the genotyped Australian group consisted of...
MODZT. Between Australian and Midwestern American populations, genomic inflation appeared to be primarily driven by a small number of highly polymorphic SNPs, while the remainder of the genome appears comparable. In a similar fashion, the results of the GWAS of Midwestern American and Dutch populations suggested moderate genetic differences between the two populations without genome-wide significant loci.

Twin families are major contributors of phenotype and genotype data to collaborative research initiatives. Twins are often motivated to take part because of information on their zygosity (Odintsova et al., 2018). The results from the work presented here are encouraging for ongoing collaborative projects, including the genetics of twinning. Collaborative efforts of the Australian, Netherlands and other twin registries have contributed to many landmark genetic studies, including the identification of two genetic variants associated with dizygotic twinning (Mbarek et al., 2016). Participation of twin cohorts and their families from geographically distinct regions such as Nigeria will undoubtedly help facilitate the elucidation of additional genetic variants underlying complex traits, including dizygotic twinning, due to the large regional differences in twinning rates (Hall, 2003; Hookestra et al., 2008; Smits & Monden, 2011).

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2019.41.

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Conflict of interest. The authors declare no conflict of interest.

Ethical standards. All participants provided informed consent. This study was conducted under Institutional Review Board approval at all study sites. Netherlands Twin Register (Dutch cohort): The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the US Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance — FWA00017598; IRB/institute codes, NTR 03-180). Avera Twin Register (Midwestern American cohort): The study was approved by the Avera Institutional Review Board and the Avera Department of Human Subject's protection.

Australia: The project was approved by the Queensland Institute of Medical Research Human Ethics Committee.

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