Replication of genome-wide association studies (GWAS) loci for fasting plasma glucose in African-Americans

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Abstract

Aims/hypothesis Chronically elevated blood glucose (hyperglycaemia) is the primary indicator of type 2 diabetes, which has a prevalence that varies considerably by ethnicity in the USA, with African-Americans disproportionately affected. Genome-wide association studies (GWASs) have significantly enhanced our understanding of the genetic basis of diabetes and related traits, including fasting plasma glucose (FPG). However, the majority of GWASs have been conducted in populations of European ancestry. Thus, it is important to conduct replication analyses in populations with non-European ancestry to identify shared loci associated with FPG across populations.

Methods We used data collected from non-diabetic unrelated African-American individuals (n=927) who participated in the Howard University Family Study to attempt to replicate previously published GWASs of FPG. Of the 29 single nucleotide polymorphisms (SNPs) previously reported, we directly tested 20 in this study. In addition to the direct test, we queried a 500 kb window centred on all 29 reported SNPs for local replication of additional markers in linkage disequilibrium (LD).

Results Using direct SNP and LD-based comparisons, we replicated multiple SNPs previously associated with FPG and strongly associated with type 2 diabetes in populations with European ancestry. The replicated SNPs included those in or near TCF7L2, SLC30A8, G6PC2, MTNR1B, DGKB-TMEM195 and GCKR. We also replicated additional variants in LD with the reported SNPs in ZMAT4 and adjacent to IRS1.

Conclusions/interpretation We identified multiple GWAS variants for FPG in our cohort of African-Americans. Using an LD-based strategy we also identified SNPs not previously reported, demonstrating the utility of using diverse populations for replication analysis.

Keywords African-American · Association · GWAS · Replication · Type 2 diabetes

Abbreviations

CEU Centre d’Etude du Polymorphisme (Utah residents with northern and western European ancestry)
FPG Fasting plasma glucose
GWAS Genome-wide association study
HUFS Howard University Family Study
LD Linkage disequilibrium
MAF Minor allele frequency
MAGIC Meta-analyses of Glucose and Insulin-related traits Consortium
SNP Single nucleotide polymorphism
YRI Yoruba in Ibadan, Nigeria
Introduction

Genome-wide association studies (GWASs) have significantly added to our understanding of the genetic basis of type 2 diabetes and related traits, including fasting plasma glucose (FPG), by identifying a number of genes potentially involved in the pathophysiology of this common complex disease [1]. However, the majority of FPG GWASs have been conducted in individuals of European descent, many of which were included in a recent meta-analysis [2]. While this information has laid an important foundation, it is important to investigate whether identified loci transfer across populations with different ancestral backgrounds [3] and whether novel variants could be identified as recently demonstrated in populations of East Asian and Indian backgrounds [4, 5]. Here, we conducted replication of published GWAS results for FPG in African-Americans from the metropolitan area of Washington DC, USA.

Methods

Ethics statement Ethical approval for the Howard University Family Study (HUFS) was obtained from the Howard University Institutional Review Board and written informed consent was obtained from each participant.

Study design The individuals studied were unrelated non-diabetic participants over the age of 20 years (n=927) enrolled in the HUFS. This population-based study of African-Americans in the Washington, DC metropolitan area has been previously described by Adeyemo et al. [6].

For the present study, participants with FPG ≥7 mmol/l or who were receiving treatment for diabetes were excluded. Additional characteristics of the cohort can be found in Electronic supplementary material (ESM) Table 1.

Genotyping All 927 DNA samples were prepared and genotyped as described by Adeyemo et al. [6]. Briefly, all samples passed a sample success rate of 95%. Single nucleotide polymorphisms (SNPs) were excluded if they had a success rate of less than 95% (41,885 SNPs excluded), a minor allele frequency (MAF) ≤0.01 (19,154 SNPs excluded), or had a p value for the Hardy-Weinberg test of equilibrium <10^{-3} (6,317 SNPs excluded). The current analysis focuses on the 808,465 autosomal SNPs that passed these filters. In addition, imputation was performed as reported by Shriner et al. [3]. We successfully imputed 1,506,100 SNPs using the Yoruba in Ibadan, Nigeria (YRI) reference panel and an additional 52,291 SNPs using the Centre d’Etude du Polymorphisme (Utah residents with northern and western European ancestry) (CEU) reference panel, for a total of 2,366,856 experimentally determined and imputed SNPs.

Statistical analyses FPG was log-transformed and values greater than ±3 SDs from the mean value were winsorised (n=8). All regression models were adjusted for age, sex, BMI and one EIGENSTRAT axis under an additive model. In separate analyses, hypertension was also adjusted for the known association with insulin resistance [7], but the effect was inconsistent, with the magnitude of the p value marginally increasing or decreasing significance for some SNPs (data not shown).

Replication analysis was performed on SNPs identified in GWASs of FPG based on information in the National Human Genome Research Institute’s catalogue of published GWASs (www.genome.gov/gwastudies/). The query returned hits indicating reported SNPs, their respective p values and associated genes (ESM Table 2). If multiple studies reported the same SNP, the SNPs with the lowest p value were included in the present study. The returned results included 16 SNPs associated with FPG in the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) study [2]. The MAGIC SNPs were supplemented by 13 additional SNPs from previously published GWASs (ESM Table 2) for a total of 29 SNPs that we attempted to replicate in our African-American cohort.

Our replication effort occurred in two stages. In the first stage, we attempted to replicate 20 of the 29 exact published SNPs (i.e. direct replication) available in the HUFS dataset. For this stage, SNPs were considered replicated if the same HUFS SNP had a p value <0.05. For the second stage, we performed a local replication analysis based on a 500 kb linkage disequilibrium (LD) block containing a query SNP determined by the SNPs most distant from the query SNP with r^2>0.3. We used the HapMap CEU LD data (http://hapmap.ncbi.nlm.nih.gov/downloads/ld_data/2008-06/00README.TXT) for all SNPs except for rs2166706 where the Gujarati Indians in Houston, Texas, USA (GIH) reference dataset (http://hapmap.ncbi.nlm.nih.gov/downloads/ld_data/2008-09_phaseIII/00README.txt) was used to match the original published GWAS population [5]. Second, we estimated the covariance matrix for the block of SNPs using the HUFS genotype data. Third, the covariance matrix was spectrally decomposed and the effective degrees of freedom, N_{eff}, were estimated using the relationship N_{eff} = \left(\sum_{k=1}^{K} \lambda_k\right) / \left(\sum_{k=1}^{K} \lambda_k^2\right), in which \lambda_k is the kth eigenvalue of the K\times K covariance matrix for the K SNPs [8]. Fourth, the nominal significance threshold \alpha=0.05 was divided by N_{eff}.

Power calculations were carried out using the Quanto software package (Version 1.2.3, http://hydra.usc.edu/gxe/).
Calculations were based on: continuous outcome; an independent individuals design; and a gene-only hypothesis. An additive inheritance model was applied for varying MAFs. MAFs were calculated based on HUFS data; for SNPs with no associated HUFS data, HapMap- or Perlegen-reported MAFs were used. The power for the present study was determined based on reported effect estimates for FPG for each reported MAGIC SNP [2].

Results

Of the 16 SNPs recently reported in the MAGIC meta-analysis of over 122,000 participants [2], 12 were available for testing in the HUFS dataset (ESM Table 2). We directly replicated three SNPs (rs2191349, rs11558471 and rs4506565) located in or near DGKB-TMEM195, SLC30A8 and TCF7L2 genes respectively (Table 1). We also replicated SNPs from other GWASs for FPG: rs2722425 within ZMAT4 (p value=0.024) as well as rs625643 (p value=0.048), which is located in a functionally unknown region on chromosome 1 (Table 1). SNPs from the remaining studies that did not directly replicate are not shown. We note that SNPs in C2CD4B, FADS1, GCK and G6PC2 from the MAGIC study and IRS1, PDE4B, and ATP8B4 from other GWASs were not directly compared in the HUFS dataset owing to quality-control filters or lack of genotyping or imputation data.

We also analysed SNPs that were in LD (r^2 ≥ 0.3) with each discovery SNP (ESM Fig. 1). This replication strategy, which queried a 500 kb window centred on the index SNP, yielded a total of 317 SNPs located in or near nine different genes or unknown gene region (G6PC2, GCKR, MTNR1B, DGKB-TMEM195, TCF7L2, SLC30A8, AK024684, ZMAT4 and IRS1). Thirty-eight SNPs distributed across all nine gene regions of the 317 SNPs tested were significantly associated with FPG after Bonferroni correction for multiple comparisons (Table 2).

Based on reported effect sizes of the 14 MAGIC loci (excluding TCF7L2 and SLC30A8), the power was calculated for each SNP using the African-American MAFs where available (ESM Table 3). The estimated power for this study ranged from a low of 0.25 to a high of 0.99. The SNPs in the four genes most strongly powered (i.e. > 90% power) in this study were either directly replicated (DGKB-TMEM195) or locally replicated (G6PC2, MTNR1B and GCKR) with markers in moderate LD (r^2 ≥ 0.4). The effect sizes for SNPs rs4506565 and rs11558471 (previously reported loci TCF7L2 and SLC30A8, respectively) were not reported in the MAGIC study.

| SNP       | Nearest gene | Region     | Position   | Reported p value | HUFS           | Allele (effect/other) | β       | p value |
|-----------|--------------|------------|------------|------------------|----------------|-----------------------|--------|---------|
| rs340874  | PROX1        | 1q41       | 212225879  | 7.1 × 10^{-8}    | C/T            | −0.0124               | 0.191  |
| rs780094  | GCKR         | 2p23.2     | 27594741   | 2.5 × 10^{-12}   | T/C            | 0.0034                | 0.66^a |
| rs11708067| ADCY5        | 3q13.33    | 124548468  | 8.7 × 10^{-9}    | G/A            | −0.0028               | 0.73^a |
| rs11920090| SLC2A2       | 3q26.2     | 172200215  | 1.9 × 10^{-6}    | A/T            | 0.0001                | 1.0    |
| rs2191349 | DGKB-TMEM195 | 7p21.2     | 15030834   | 7.8 × 10^{-17}   | G/T            | −0.0136               | 0.023^b|
| rs11558471| SLC30A8      | 8q24.11    | 118254914  | 2.6 × 10^{-11}   | G/A            | −0.0255               | 0.0074^b|
| rs7034200 | GLIS3        | 9p24.2     | 4279050    | 1.2 × 10^{-4}    | C/A            | 0.0056                | 0.35   |
| rs10885122| ADRA2A       | 10q25.2    | 113032083  | 8.4 × 10^{-11}   | G/T            | 0.0079                | 0.2    |
| rs4506565 | TCF7L2       | 10q25.2    | 114746031  | 1.2 × 10^{-8}    | T/A            | −0.0117               | 0.036^b|
| rs10830963| MTNR1B       | 11q21      | 92348358   | 1.2 × 10^{-68}   | G/C            | −0.0046               | 0.67^a |
| rs11605924| CRY2         | 11p11.2    | 45829667   | 1.5 × 10^{-9}    | C/A            | 0.0048                | 0.57   |
| rs7944584 | MADD         | 11p11.2    | 47292896   | 1.5 × 10^{-9}    | T/A            | 0.0026                | 0.84^a |

From other FPG GWASs

rs625643 AK024684 1p32.3 54409755 4.0 × 10^{-3} T/C 0.01141 0.05^a,b
rs2722425 ZMAT4 8p11.21 40603396 9.0 × 10^{-6} T/C 0.01326 0.024^b

^a Genotyped HUFS SNPs (all other SNPs were imputed)
^b SNPs from reported studies that are also statistically significant (p<0.05) in the HUFS dataset.
Table 2 Significant SNPs and their effects ($\beta$) after Bonferroni correction that were in LD ($r^2 \geq 0.3$) with reported SNPs from the MAGIC study and other GWASs of FPG

| Region | Nearest gene | Reported SNP in LD | Study results |
|--------|--------------|-------------------|---------------|
|        |              | SNP p value | Allele (effect/other) | $\beta$ | $r^2$ | p value | df | Corrected p value |
| HUFS   |              |              |                  |          |      |        |     |                  |
| 2q31.1 | G6PC2        | rs560887 | $4.4 \times 10^{-35}$ | rs575671 | A/G | -0.0185 | 0.50 | 0.003 | 2.76 | 0.01 |
|        |              | rs780100 | $2.5 \times 10^{-12}$ | rs704791 | T/C | 0.0142 | 0.46 | 0.020 | 2.55 | 0.05 |
|        |              | rs704791 |                     | rs704791 | T/C | 0.0142 | 0.46 | 0.020 | 2.55 | 0.05 |
|        |              | rs704795 |                     | rs704795 | G/A | 0.0154 | 0.46 | 0.011 | 2.55 | 0.03 |
|        |              | rs10208529|                   | rs10208529| T/A | -0.0198 | 0.54 | 0.021 | 2.55 | 0.05 |
| 7p21.2 | DGKB-TMEM195 | rs2191349 | $7.8 \times 10^{-17}$ | rs2215383 | T/C | -0.0175 | 1.0  | 0.003 | 2.38 | 0.007 |
|        |              | rs10244051|                    | rs10244051| T/G | -0.0163 | 1.0  | 0.004 | 2.38 | 0.01 |
|        |              | rs10258074|                    | rs10258074| A/T | -0.0163 | 0.97 | 0.004 | 2.38 | 0.001 |
|        |              | rs2191348 |                    | rs2191348| G/T | -0.0164 | 1.0  | 0.004 | 2.38 | 0.009 |
|        |              | rs6947830 |                    | rs6947830| G/A | -0.0131 | 1.0  | 0.022 | 2.38 | 0.05^b |
|        |              | rs4719433 |                    | rs4719433| T/C | -0.0131 | 1.0  | 0.022 | 2.38 | 0.05^b |
|        |              | rs1974620 |                    | rs1974620| C/T | -0.0147 | 0.97 | 0.011 | 2.38 | 0.03^b |
|        |              | rs1558317 |                    | rs1558317| T/A | -0.0147 | 0.96 | 0.011 | 2.38 | 0.03 |
|        |              | rs1558318 |                    | rs1558318| T/A | -0.0147 | 0.97 | 0.011 | 2.38 | 0.03 |
| 8q24.11| SLC30A8     | rs11558471| $2.6 \times 10^{-11}$ | rs11774700| C/T | -0.0249 | 0.78 | 0.004 | 1.74 | 0.006 |
| 10q25.2| TCF7L2      | rs4506565 | $1.2 \times 10^{-8}$ | rs7901695| C/T | -0.0127 | 0.88 | 0.022 | 1.72 | 0.04 |
| 11q21 | MTNR1B      | rs10830963| $1.2 \times 10^{-68}$ | rs1447350| C/G | -0.0141 | 0.43 | 0.014 | 2.46 | 0.03 |
|        |              | rs1447351 |                    | rs1447351| A/G | -0.0131 | 0.43 | 0.022 | 2.46 | 0.05 |
|        |              | rs4611171 |                    | rs4611171| T/G | -0.0131 | 0.42 | 0.022 | 2.46 | 0.05^b |
|        |              | rs4645008 |                    | rs4645008| A/C | -0.0131 | 0.42 | 0.022 | 2.46 | 0.05 |
|        |              | rs4406791 |                    | rs4406791| A/C | -0.0131 | 0.42 | 0.022 | 2.46 | 0.05 |
|        |              | rs1447352 |                    | rs1447352| G/A | -0.0131 | 0.43 | 0.022 | 2.46 | 0.05 |
|        |              | rs9666752 |                    | rs9666752| G/A | -0.0148 | 0.36 | 0.009 | 2.46 | 0.02 |
|        |              |              |                        |               |      |        |     |                  |        |
| From other FPG GWASs |              |              |                        |               |      |        |     |                  |        |
| 1p32.1 | AK024684    | rs625643 | $4 \times 10^{-3}$  | rs671431| G/C | 0.0143 | 0.62 | 0.022 | 1.66 | 0.04 |
| 2q36.1 | IRS1        | rs2943641 | $5 \times 10^{-12}$ | rs6713510| A/T | 0.0169 | 0.43 | 0.005 | 5.13 | 0.03 |
|        |              | rs4072096 |                    | rs4072096| G/A | 0.0171 | 0.43 | 0.005 | 5.13 | 0.03 |
|        |              | rs4645008 |                    | rs4645008| A/C | 0.0181 | 0.43 | 0.003 | 5.13 | 0.02 |
|        |              | rs2943644 |                    | rs2943644| T/C | 0.0181 | 0.41 | 0.003 | 5.13 | 0.02 |
|        |              | rs2943633 |                    | rs2943633| C/T | 0.0167 | 0.45 | 0.005 | 5.13 | 0.03 |
|        |              | rs2972150 |                    | rs2972150| C/A | 0.0161 | 0.45 | 0.007 | 5.13 | 0.04 |
|        |              | rs2943636 |                    | rs2943636| C/T | 0.0165 | 0.45 | 0.006 | 5.13 | 0.03 |
|        |              | rs2972149 |                    | rs2972149| A/G | 0.0201 | 0.50 | 0.0007 | 5.13 | 0.004^b |
|        |              | rs1515114 |                    | rs1515114| A/T | 0.0196 | 0.60 | 0.0009 | 5.13 | 0.005^b |
|        |              | rs2722429 |                    | rs2722429| G/A | 0.0186 | 0.60 | 0.001 | 5.13 | 0.006 |
| 8p11.21| ZMAT4       | rs2722425 | $9 \times 10^{-5}$  | rs1823643| T/C | 0.0192 | 0.89 | 0.002 | 1.53 | 0.007 |
|        |              | rs1823643 |                    | rs1823643| C/A | 0.0145 | 0.65 | 0.021 | 1.53 | 0.03^b |
|        |              | rs1823643 |                    | rs1823643| C/T | 0.0146 | 0.68 | 0.018 | 1.53 | 0.03 |

a df calculated from covariance matrix of SNPs in LD ($r^2 \geq 0.3$) with query SNP in 500 kb window; df were used for Bonferroni corrections
b Genotyped HUFS SNPs (all other SNPs were imputed)
Discussion

Chronically elevated FPG is a primary indicator of diabetes, making it an important biomarker of the progression of impaired glucose metabolism. In this paper, we attempted to replicate, in nearly 1,000 African-Americans, significant GWAS loci for FPG in populations of predominantly European ancestry. However, this study had over 80% power to replicate similar effect sizes for 10 of the 14 SNPs reported by the MAGIC study. The need for understanding differential susceptibility to diseases at the population level makes the identification of risk factors for diabetes and its indicators, including FPG, particularly important in the African-American community and other ethnic groups, given their disproportionate rate of morbidity and mortality from diabetes and associated complications. Unfortunately and for multiple reasons, the majority of GWASs aimed at identifying genetic variants associated with FPG and diabetes have so far focused predominantly on individuals of European ancestry. While the results from these studies provided tremendous insight into the genetic architecture of the disease, recent studies of non-European populations have shown utility in expanding the breadth of populations studied. Specifically, studies in East Asians allowed for a ‘wider net’ to be cast in the identification of type 2 diabetes susceptibility variants. The present study’s focus on individuals self-identified as African-Americans not only widens the net but also underscores the need for directed investigation of under-represented populations.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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