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ARTICLE

Common genetic variants differentially influence the transition from clinically defined states of fasting glucose metabolism

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Abstract
Aims/hypothesis Common genetic variants have been associated with type 2 diabetes. We hypothesised that a subset of these variants may have different effects on the transition from normal fasting glucose (NFG) to impaired fasting glucose (IFG) than on that from IFG to diabetes.
Methods We identified 16 type 2 diabetes risk variants from the Illumina Broad Candidate-gene Association Resource (CARe) array genotyped in 26,576 CARe participants. Participants were categorised at baseline as NFG, IFG or type 2 diabetic (n=16,465, 8,017 or 2,291, respectively). Using Cox proportional hazards and likelihood ratio tests (LRTs), we compared rates of progression by genotype for 4,909 (NFG to IFG) and 1,518 (IFG to type 2 diabetes) individuals, respectively. We then performed multinomial regression analyses at baseline, com-

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paring the risk of assignment to the NFG, IFG or diabetes groups by genotype.
Results The rate of progression from NFG to IFG was significantly greater in participants carrying the risk allele at *MTNR1B* ($p=1\times10^{-4}$), nominally greater at *GCK* and *SLC30A8* ($p<0.05$) and nominally smaller at *IGF2BP2* ($p=0.01$) than the rate of progression from IFG to diabetes by the LRT. Results of the baseline, multinomial regression model were consistent with these findings.
Conclusions/interpretation Common genetic risk variants at *GCK*, *SLC30A8*, *IGF2BP2* and *MTNR1B* influence to different extents the development of IFG and the transition from IFG to type 2 diabetes. Our findings may have implications for understanding the genetic contribution of these variants to the development of IFG and type 2 diabetes.

Keywords Common genetic variants · Diabetes mellitus · Genetics · Glycaemic progression · Impaired fasting glucose · Normal fasting glucose · Single nucleotide polymorphism · Type 2 diabetes

Abbreviations

ARIC	Atherosclerosis Risk in Communities
CARDIA	Coronary Artery Risk Development in Young Adults
CARe	Candidate-gene Association Resource
CEU	Centre d'Etude du Polymorphisme (Utah residents with ancestry from Northern and Western Europe)
CHS	Cardiovascular Health Study
DIAGRAM	Diabetes Genetics Replication and Meta-analysis
FHS	Framingham Heart Study
IBC Chip	Illumina Broad CARe iSelect
IFG	Impaired fasting glucose
LRT	Likelihood ratio test
MAGIC	Meta-Analysis of Glucose and Insulin-related Traits Consortium
MESA	Multi-Ethnic Study of Atherosclerosis
NFG	Normal fasting glucose
SNP	Single nucleotide polymorphism
YRI	Yoruba from Ibadan, Nigeria

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Introduction

Impaired fasting glucose (IFG) is a clinically defined prediabetic state, yet only 25% of individuals with IFG progress to type 2 diabetes mellitus over 3 to 5 years [1]. Evaluation of genetic information may elucidate biological pathways that contribute to the development of IFG and to the clinical transition from IFG to type 2 diabetes.

More and more single nucleotide polymorphisms (SNPs) have been associated unequivocally with increased risk of type 2 diabetes [2]. Recent work by the Meta-Analysis of Glucose and Insulin-related Traits Consortium (MAGIC) and the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) investigators has identified overlap, in cross-sectional examination, between loci that influence type 2 diabetes risk and levels of non-diabetic fasting glucose [3–5]. Longitudinal studies from other groups have identified loci at *GCK* [6] and *MTNR1B* [7] that are associated with increased measures of non-diabetic fasting glycaemia and type 2 diabetes risk.

However, results from the MAGIC and DIAGRAM groups have shown that loci associated with a comparable risk of type 2 diabetes have disproportionate associations with elevations in fasting glucose [3, 4]. In addition, the locus with the strongest effect on type 2 diabetes (*TCF7L2*) has only a modest effect on fasting glucose, whereas one of the loci with the strongest effect on fasting glucose (*G6PC2*) has a negligible effect on type 2 diabetes [3, 4]. More recently, investigators have demonstrated that loci influencing type 2 diabetes risk and fasting glucose levels at baseline are associated with non-significant increases in fasting glucose over 10 years [8], while others have shown that the association between fasting glucose and genetic variants that influence fasting glucose are stable over time, similarly to age-related changes in non-diabetic individuals [9].

Together, these findings raise the hypothesis that a subset of common type 2 diabetes risk variants may strongly promote the development of non-diabetic elevations in fasting

glucose, i.e. the transition from normal fasting glucose (NFG) to IFG, but weakly influence the development of type 2 diabetes, i.e. the transition from IFG to type 2 diabetes. To test this hypothesis, we performed analyses within the Candidate-gene Association Resource (CARe) [10]. This consortium comprises nine National Heart Lung Blood Institute (NHLBI)-supported cohort studies, which have accrued longitudinal clinical measurements, including cardiovascular and glycaemic traits, in approximately 50,000 participants; all participants have been genotyped in a custom-made Illumina Broad CARe iSelect (IBC Chip) array [11].

Methods

Study participants and clinical characteristics From the nine cohorts within CARe [10], longitudinal fasting glucose measures were available in Atherosclerosis Risk in Communities (ARIC) [12], Coronary Artery Risk Development in Young Adults (CARDIA) [13], Cardiovascular Health Study (CHS) [14], Framingham Heart Study (FHS) [15] and Multi-Ethnic Study of Atherosclerosis (MESA) [16]. Protocols for CARe were approved by local ethics committees and/or institutional review boards of each participating recruitment or analysis site. Informed consent was obtained from all study participants.

Glycaemic phenotypes We used the ADA criteria [17] to categorise participants as NFG (<5.6 mmol/l), IFG (5.6–6.9 mmol/l) or type 2 diabetic (≥7.0 mmol/l) based on the measurements within CARe. Participants who, despite having a fasting glucose measurement within the expected range, had been otherwise identified at the time of measurement as having diabetes or taking medications to treat diabetes (oral medications or insulin) were included and analysed in the type 2 diabetes group. OGTT results, if available at the time of the fasting measurement, were used only to re-assign participants to the type 2 diabetes group on the basis of a 2 h OGTT measurement ≥11.1 mmol/l.

Measurements Measurements of fasting glucose were performed as previously described in ARIC [18], CARDIA [19], CHS [20], FHS [21, 22] and MESA [23]; all were conducted 8 to 12 h after the last meal. Follow-up fasting glucose measurements occurred on average at 3, 6 and 9 years after baseline for ARIC, at 3 and 8 years after baseline for CARDIA, at 3 and 7 years after baseline for CHS, at 4, 8, 12 and 16 years after baseline for FHS, and at 2, 4 and 6 years after baseline for MESA.

SNP selection and genotyping Genotyping had been performed previously on the custom-made IBC Chip [11] and the genotype list is publicly available [24]. The IBC chip was designed in 2007 and included type 2 diabetes-associated SNPs identified up until that time (including early access to some findings published later) [25–28]. Tag SNPs were selected as previously described [11]. Approximately 99.5% concordance was observed against HapMap data, and SNPs failing Hardy–Weinberg equilibrium ($p<0.01$) were excluded. To optimise capture of the more recently identified type 2 diabetes risk SNPs, we used the SNP Annotation and Proxy Search (SNAP) program [29] to identify the best proxies within the CARe genotype database, with a lower linkage disequilibrium threshold of $r^2=0.8$. The linkage disequilibrium was estimated using the Centre d'Etude du Polymorphisme (Utah residents with ancestry from Northern and Western Europe) (CEU) for European-American samples and the Yoruba from Ibadan, Nigeria (YRI) for African-American samples (HapMap release 21 [30]), as used in the design of the IBC chip

Statistical analysis We identified all SNPs or their proxies in CARe that had previously been associated with type 2 diabetes at genome-wide significance ($p<5\times10^{-8}$). We validated the reported type 2 diabetes associations in CARe by estimating allelic ORs of assignment to the type 2 diabetes versus the NFG groups. We meta-analysed the results separately for European-American and African-American participants, using the Cochran–Mantel–Haenszel method for each risk allele and type 2 diabetes versus NFG group allocation at the baseline measurement. Because a detectable association signal with increased type 2 diabetes risk is a necessary prerequisite for statistical comparison of the transition from NFG to IFG with that from IFG to type 2 diabetes, we selected for further analysis only those risk variants that were associated with at least a nominally significant ($p<0.05$) increase in type 2 diabetes risk in CARe. Power to detect type 2 diabetes risk in the European-American and African-American participants in CARe was determined with the Genetic Power Calculator (http://ibgwww.colorado.edu/~pshaun/gpc/) [31].

For the 11 risk variants that were associated with increased risk of type 2 diabetes in CARe, we fitted standard Cox regression models to compare the rate of progression for individuals at transition from NFG to IFG (3,836 European-American and 1,073 African-American participant events) and from IFG to type 2 diabetes (1,060 European-American and 458 African-American participant events) based on the presence of risk alleles. Individuals who progressed from NFG to type 2 diabetes (109 European-Americans, 80 African-Americans) during a single observation period were excluded from the longitudinal analyses, as it was not possible to parse the rate from NFG to IFG or IFG to type 2 diabetes. Individuals who regressed from IFG to NFG were included in longitudinal analyses examining further progressions to type 2 diabetes

(i.e. IFG to type 2 diabetes), but were not included in longitudinal analyses examining subsequent NFG to IFG transitions (i.e. return to IFG status). Participants categorised as type 2 diabetes were included in the type 2 diabetes group at all subsequent time points, regardless of their follow-up fasting blood sugar values. Therefore, participants in the type 2 diabetes group at the baseline measurement did not contribute to the longitudinal analyses. All data were analysed together and a covariate for each cohort was included in the analysis. Time to progression was determined by subtracting the participant's age at baseline measurement from age at the follow-up measurement. We calculated HRs for each risk allele, adjusting these analyses for age at baseline and sex.

Because the sample size for transition from NFG to IFG and from IFG to type 2 diabetes differed, we directly compared the effects of type 2 diabetes risk alleles at the two clinical transitions using a likelihood ratio test (LRT). For the LRT, we used a one-degree of freedom χ^2 test, which compared the maximum likelihood of a risk allele being associated with the HR averaged across the first and second transitions against a model with the first and second transition HRs. The null hypothesis for the LRT was that the HR of either clinical transition was the same as the average HR of two transitions. A significant finding indicated that the HR at one transition was different from the HR at the other clinical transition. As the IFG group was present in both clinical transitions, we were able to compare the two HRs in a nested model. Inter-cohort heterogeneity was tested by calculating the maximum likelihood values for the HR of each clinical transition per cohort and comparing each cohort HR with the HR of the other cohorts by a 4 degrees of freedom χ^2 analysis.

We then used multinomial logistic regression to examine the association of risk alleles with the NFG versus the IFG groups (12,480 and 6,251 European-American participants, and 3,985 and 1,766 African-American participants, respectively) and with the IFG versus the type 2 diabetes groups (6,251 and 1,422 European-American participants, and 1,766 and 869 African-American participants, respectively) at the baseline measurement of fasting glucose in CARe. Participants were grouped according to their baseline measurements, irrespective of later fasting glucose measurements. These analyses generated ORs adjusted for age at baseline and sex; all participants with baseline fasting glucose measurements were included in the analyses. A Wald test was then used to compare the two ORs at the baseline measurement.

Results

We determined the influence of known type 2 diabetes risk alleles on the transition from NFG to IFG and from IFG to type 2 diabetes in 20,153 European-American and 6,423 African-American CARe participants (Table 1). Categorisation of fasting glucose groups by cohort is provided in the Electronic supplementary material (ESM) Table 1. At the baseline measurement of fasting glucose, 12,480 European-American and 3,985 African-American participants were classified as NFG, 6,251 and 1,766 respectively as IFG, and 1,422 and 869 respectively as type 2 diabetic. There were 1,060 European-American and 458 African-American incident cases of diabetes over 7.7±2.1 years (mean±SD) of follow-up on average. Of the participants with NFG at baseline and follow-up data available, 31% (3,836 of 12,480) of European-Americans and 27% (1,073 of 3,985) of African-Americans progressed to IFG. Of the participants with IFG and follow-up data available, 15% (923 of 6,251) of European-Americans and 23% (413 of 1,766) of African-Americans progressed to type 2 diabetes by the last measure of fasting glucose. All participants categorised as type 2 diabetic at baseline were analysed in the type 2 diabetes group at all follow-up measurements. A single OGTT measurement was available for 14,445 participants, and more than one OGTT measurement was available for 9,307 participants. When an OGTT measurement was available at the time of fasting glucose measurement, 4% of all participants otherwise assigned to the NFG or IFG

Table 1 Demographic data for CARe participants per baseline glycaemic categories

Variable per ethnic group	Baseline group			Total
	NFG	IFG	Diabetes	
European-American				
n	12,480	6,251	1,422	20,153
NFG to IFG (*n*)	3,836	–	–	3,836
IFG to type 2 diabetes (*n*)	137	923	–	1,060
Men (*n*)	5,027	3,442	768	
Women (*n*)	7,408	2,802	649	
Sex not specified (*n*)	45	7	5	
FPG (mmol/l)	5.0±0.7	6.0±0.4	10.0±3.5	
BMI (kg/m^2)	26±5	28±5	30±6	
Age (years)	54±14	59±11	63±11	
African-American				
n	3,985	1,766	869	6,423
NFG to IFG (*n*)	1,073	–	–	1,073
IFG to type 2 diabetes (*n*)	45	413	–	458
Men (*n*)	1,527	762	342	
Women (*n*)	2,458	1,004	527	
FPG (mmol/l)	5.0±1.3	6.0±0.4	11.3±4.4	
BMI (kg/m^2)	28±6	30±6	32±6	
Age (years)	48±17	54±12	58±9	

Values are mean ± SD unless indicated otherwise

FPG, fasting plasma glucose

groups (1,063 of 24,431) at the first measure of fasting glucose and 1.4% of all participants otherwise assigned to the NFG or IFG groups (312 of 22,096) at the last measure of fasting glucose had overt diabetes by OGTT; these participants were reclassified as belonging to the type 2 diabetes group to ensure appropriate risk estimates for participants with known diabetes.

Of the 38 published type 2 diabetes signals, we identified 16 risk SNPs or their proxies in the CARe genotype database (Table 2). Based on our pre-specified criteria for inclusion in the study, i.e. demonstrating nominal association with type 2 diabetes in our cohort, we selected 11 SNPs in European-American participants and one in African-American participants for further analyses.

The results of the LRT for the Cox proportional hazards model analyses are provided in Table 3. In European-American participants, those who carried the common type 2 diabetes variant at *MTNR1B* had a significantly higher risk of transition from NFG to IFG than from IFG to type 2 diabetes during the observed follow-up period ($p=1\times10^{-4}$). Participants carrying the common risk variants at *GCK* and *SLC30A8* had a nominally higher risk of transition from NFG to IFG than from IFG to type 2 diabetes during the observed follow-up ($p<0.05$ for all). Participants carrying the risk variant at *IGF2BP2* had a nominally higher risk of transition from IFG to type 2 diabetes than from NFG to IFG during the observed follow-up ($p=0.01$). Participants carrying the other genotyped common risk variants had an equal risk of transition from NFG to IFG and from IFG to type 2 diabetes. Interestingly, participants carrying the risk allele at *GCKR* had a statistically positive OR for transition from NFG to IFG (OR 1.02–1.12, $p=3\times10^{-3}$), but not for transition from IFG to diabetes (0.91–1.09, $p=0.89$). Those carrying the risk allele at *TCF7L2* had a statistically positive OR for transition from NFG to IFG (1.02–1.13, $p=4\times10^{-3}$) and from IFG to diabetes (1.11–1.33, $p=1.5\times10^{-5}$). However, in both of the above instances, the association of the risk allele with the NFG to IFG transition was not significantly different from the IFG to diabetes transition in the longitudinal analyses. The test for inter-cohort heterogeneity was unremarkable for all variants in European-American participants (χ^2 analysis, $p>0.05$) (Table 3).

Comparisons between allocation to the IFG versus the NFG group and to the type 2 diabetes versus the IFG group at baseline in European-American participants are displayed in Table 4. The OR for the IFG versus NFG comparison was nominally significant ($p<0.05$) and greater than 1 for all variants examined, except at *WFS1*, *IGF2BP2* and *KCNQ1*. The OR for the type 2 diabetes versus IFG comparison was nominally significant and greater than 1 for variants at *TCF7L2*, *WFS1* and *IGF2BP2*, and nominally significant, but less than 1 at *MTNR1B*. The Wald test was significant for variants at *GCK*, *GCKR* and *MTNR1B* ($p<0.005$, all indicating a greater effect of the risk allele for the IFG versus NFG comparison than for the type 2 diabetes versus IFG comparison), and at *TCF7L2* ($p<0.001$, indicating a greater effect for the type 2 diabetes

Table 2 Selection of risk variants in the CARe IBC chip

Gene region	CARe SNP	Risk allele	CEU (r^2)[a]	YRI (r^2) [a]	MAF	OR (EA)[b]	*p* value	OR (AA)[b]	*p* value
NOTCH2	rs835574	T (min)	0.9	–	0.13	1.19	5×10^{-3}	–	–
TCF7L2	rs7903146	T (min)	1.0	1.00	0.29	1.40	2×10^{-14}	1.33	1×10^{-6}
GCKR	rs780094	C (maj)	1.0	1.00	0.42	1.27	5×10^{-8}	1.09	0.23
CDKAL1	rs7754840	C (min)	1.0	–	0.31	1.17	4×10^{-4}	–	–
GCK	rs6975024	C (min)	1.0	1.00	0.17	1.19	2×10^{-3}	1.01	0.91
KCNJ11	rs5215	C (min)	1.0	1.00	0.37	1.09	0.06	0.98	0.85
WFS1	rs5018647	C (maj)	1.0	1.00	0.40	1.11	0.02	0.94	0.32
HHEX	rs5015480	C (maj)	1.0	–	0.41	1.10	0.03	–	–
IGF2BP2	rs4402960	T (min)	1.0	1.00	0.32	1.20	3×10^{-5}	1.11	0.06
IRS1	rs2943634	C (maj)	0.9	–	0.33	1.07	0.14	1.03	0.56
KCNQ1	rs231362	G (maj)	1.0	1.00	0.49	1.14	2×10^{-3}	1.12	0.08
PPARG	rs1801282	C (maj)	1.0	–	0.12	1.13	0.06	–	–
SLC30A8	rs13266634	C (maj)	1.0	–	0.32	1.18	3×10^{-4}	–	–
HNF1A	rs12427353	G (maj)	0.8	–	0.20	1.04	0.41	–	–
MTNR1B	rs10830963	G (min)	1.0	1.00	0.26	1.19	2×10^{-4}	1.06	0.62
CDKN2A/2B	rs10811661	T (maj)	1.0	–	0.18	1.11	0.06	–	–

AA, African-American; EA, European-American; MAF, minor allele frequency in CARe participants; Maj, major allele; Min, minor allele

[a] Linkage disequilibrium metric in the named HapMap populations for the CARe SNP correlated with the published type 2 diabetes risk allele

[b] Type 2 diabetes-associated OR in CARe participants as indicated

Table 3 Longitudinal analysis results for European-American participants

Gene region	CARe SNP	NFG to IFG		IFG to type 2 diabetes		LRT		Heterogeneity[a]	
		HR (95% CI) [b]	*p* value	HR (95% CI) [b]	*p* value	χ^2	*p* value	χ^2	*p* value
NOTCH2	rs835574	1.03 (0.97, 1.11)	0.35	0.98 (0.86, 1.11)	0.73	1.11	0.29	1.04	0.90
TCF7L2	rs7903146	1.07 (1.02, 1.13)	4×10^{-3}	1.22 (1.11, 1.33)	1.5×10^{-5}	3.63	0.06	2.93	0.57
GCKR	rs780094	1.07 (1.02, 1.12)	3×10^{-3}	0.99 (0.91, 1.09)	0.89	1.9	0.17	1.94	0.75
CDKAL1	rs7754840	1.02 (0.97, 1.07)	0.49	1.11 (1.02, 1.21)	0.02	1.53	0.22	0.48	0.98
GCK	rs6975024	1.12 (1.06, 1.19)	1×10^{-4}	0.98 (0.88, 1.09)	0.68	6.56	0.01	0.32	0.99
WFS1	rs5018647	1.00 (0.95, 1.05)	0.97	1.01 (0.93, 1.10)	0.80	0.33	0.57	1.37	0.85
HHEX	rs5015480	0.99 (0.95, 1.04)	0.82	1.05 (0.97, 1.15)	0.24	0.75	0.39	2.08	0.72
IGF2BP2	rs4402960	1.00 (0.95, 1.05)	0.87	1.17 (1.07, 1.28)	4.4×10^{-4}	6.79	0.01	1.38	0.85
KCNQ1	rs231362	0.95 (0.91, 0.99)	0.03	1.04 (0.95, 1.13)	0.40	1.55	0.21	1.39	0.85
SLC30A8	rs13266634	1.08 (1.03, 1.14)	2×10^{-3}	0.99 (0.90, 1.08)	0.78	5.08	0.02	2.72	0.61
MTNR1B	rs10830963	1.20 (1.15, 1.27)	3×10^{-13}	0.98 (0.89, 1.07)	0.62	14.98	1×10^{-4}	1.16	0.89

[a] Inter-cohort heterogeneity by risk allele

[b] From the Cox proportional models

versus IFG comparison than for the IFG versus NFG comparison). The Wald test bordered on nominal significance (p=0.05) for the variant at *IGF2BP2* (suggesting a greater effect for the type 2 diabetes versus IFG comparison than for the IFG versus NFG comparison).

We tested the performance of the type 2 diabetes risk allele at *TCF7L2* in longitudinal and multinomial baseline regression models in African-American participants (ESM Table 2). For African-American participants carrying this risk allele, there was an equal rate of transition from NFG to IFG and from IFG to type 2 diabetes. The test for inter-cohort heterogeneity was unremarkable. In the baseline analysis, the OR for the type 2 diabetes versus IFG comparison was significant ($p=6\times10^{-5}$) and the Wald test was significant ($p=5\times10^{-4}$, indicating a greater effect for the type 2 diabetes versus IFG comparison than for the IFG versus NFG comparison).

Discussion

In the present study, we found that in participants carrying common type 2 diabetes risk alleles at *GCK*, *SLC30A8* and *MTNR1B*, the rate of progression from NFG to IFG was higher than that from IFG to type 2 diabetes, while in those carrying the common risk allele at *IGF2BP2*, progression from IFG to type 2 diabetes occurred at a higher rate than progression from NFG to IFG. These longitudinal observations were consistent with those in the baseline multinomial regression model. In the presence of the *GCK* and *MTNR1B*

Table 4 Multinomial baseline regression model results for European-American participants

Gene region	CARe SNP	IFG vs NFG (referent)		Type 2 diabetes vs IFG (referent)		Wald test	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value	Statistic	*p* value
NOTCH2	rs835574	1.10 (1.03,1.17)	0.01	1.02 (0.91,1.13)	0.77	1.48	0.22
TCF7L2	rs7903146	1.09 (1.04,1.15)	4×10^{-4}	1.27 (1.18,1.37)	7×10^{-10}	10.67	0.001
GCKR	rs780094	1.19 (1.14,1.25)	8×10^{-14}	1.04 (0.97,1.12)	0.30	9.5	0.002
CDKAL1	rs7754840	1.09 (1.03,1.14)	0.001	1.05 (0.97,1.13)	0.25	0.68	0.41
GCK	rs6975024	1.23 (1.16,1.30)	6×10^{-12}	0.95 (0.87,1.05)	0.32	20.59	6×10^{-6}
WFS1	rs5018647	1.01 (0.96,1.06)	0.69	1.09 (1.01,1.17)	0.02	2.95	0.09
HHEX	rs5015480	1.08 (1.03,1.13)	0.001	1.03 (0.98,1.14)	0.36	1.04	0.31
IGF2BP2	rs4402960	1.04 (0.99,1.09)	0.11	1.14 (1.06,1.23)	0.001	3.86	0.05
KCNQ1	rs231362	1.03 (0.99,1.08)	0.14	1.06 (0.98, 1.14)	0.13	0.26	0.61
SLC30A8	rs13266634	1.11 (1.05,1.16)	5×10^{-5}	1.04 (0.96, 1.12)	0.35	1.84	0.17
MTNR1B	rs10830963	1.35 (1.28,1.42)	5×10^{-31}	0.88 (0.81,0.95)	0.001	79.63	5×10^{-19}

risk alleles, the odds of a participant being in the IFG versus the NFG group were greater than the odds of being in the type 2 diabetes versus the IFG group, whereas in the presence of the *IGF2BP2* risk allele, the odds of a participant being in the type 2 diabetes versus the IFG group were greater than the odds of being in the IFG versus the NFG group. While participants carrying the risk allele at *SL30A8* were significantly more likely to be in the IFG than the NFG group, they were as likely to be in the IFG as in the type 2 diabetes groups in the baseline analyses. These results suggest that the biological effect of type 2 diabetes risk alleles at *GCK*, *SLC30A8* and *MTNR1B* may be more important for the development of prediabetic fasting hyperglycaemia than for overt diabetes, and that the risk allele at *IGF2BP2* may be more important for the progression from IFG to type 2 diabetes than for that from NFG to IFG.

Notably, participants carrying the common type 2 diabetes risk alleles at *GCKR* and *TCF7L2* progressed at statistically equal rates from NFG to IFG and from IFG to type 2 diabetes in the longitudinal analyses, suggesting that these risk loci are active in the development of IFG and overt diabetes. However, the ORs in the longitudinal analyses and the results of the baseline multinomial analyses indicate that the *GCKR* locus may be more associated with the transition from NFG to IFG than with the development of overt diabetes, and that the *TCF7L2* locus may be more associated with the development of overt diabetes from IFG. These discrepancies merit further investigation in European and African populations.

Our study has high statistical power, a large number of participants and longitudinal measurement of fasting glucose over time, as well as using clinically defined measures of glycaemia. Nevertheless, we acknowledge several limitations.

First, relatively few participants in CARe have longitudinal OGTT data, a measure sometimes used in clinical practice for the diagnosis of diabetes and prediabetic states [32, 33]. In a setting of sufficient longitudinal OGTT data and adequate statistical power, a parallel set of analyses using OGTT classification alone and in combination with fasting glucose measurements could be performed to categorise study participants.

Second, of the known type 2 diabetes risk alleles, genotype information and nominal association with type 2 diabetes risk in CARe were available for 16 SNPs only in European-American participants and nine SNPs only in African-American participants. This is partly the result of the rapid discovery of new type 2 diabetes loci since the development of the IBC chip in 2007. Updated genotyping arrays and their deployment across these same cohorts might allow more risk alleles to be incorporated into similar analyses in the future.

Third, we had limited power for detection of type 2 diabetes risk in African-American participants in CARe (ESM Table 3). This stems from the relatively smaller numbers of African-American participants and the less well defined linkage disequilibrium architecture around risk variants in African populations. Replication of these findings in African-American and other non-European populations will be possible in the near future, as improved understanding of type 2 diabetes genetic architecture in multi-ethnic groups and updated genotype arrays incorporating more ancestry-informative SNPs allow the correction of genetic admixture.

While the findings of our study are novel, they expand logically from the existing literature. The type 2 diabetes risk alleles at *GCK*, *SLC30A8* and *MTNR1B* have been previously associated with an increased cross-sectional and longitudinal risk of type 2 diabetes and elevations in fasting glucose in non-diabetic individuals [3, 4, 6–8, 34–36]. Interestingly, these risk alleles are all associated with age-related trajectories in fasting glucose, similarly to those in non-diabetic individuals [9]. Our results are consistent with these findings, demonstrating that type 2 diabetes risk loci at *GCK*, *SLC30A8* and *MTNR1B* increase the risk of prediabetic fasting glucose levels, but may have minimal influence on the clinical transition from IFG to type 2 diabetes. The type 2 diabetes risk allele at *IGF2BP2* has also been associated with increased cross-sectional risk of type 2 diabetes and fasting glucose elevations in non-diabetic individuals [37], but its association with longitudinal changes in glucose has not been studied. We observed the strongest effects in our models at the *MTNR1B* risk allele. Interestingly, this risk allele has recently been shown to be associated with increased fasting glucose values, but decreased 2 h OGTT values over a 10 year observation period [8], a finding that may be consistent with the seemingly protective influence of the *MTNR1B* locus for transition from IFG to type 2 diabetes in our study.

The mechanisms by which common variants in *GCK*, *SLC30A8*, *IGF2BP2* and *MTNR1B* exert different degrees of influence on the transition from NFG to IFG and from IFG to type 2 diabetes are unknown. It is notable, however, that mutations in the glucokinase gene impair pancreatic beta cell glucose-sensing, so that individuals with heterozygous mutations develop MODY and classically experience non-progressive hyperglycaemia [38]. Further work may illuminate whether common mutations in *GCK*, as well as *SLC30A8* and *MTNR1B* (which are known to influence beta cell function [35, 36, 39]), and possibly *GCKR* (which encodes a glucokinase regulatory protein [40]) share common mechanisms in the development of type 2 diabetes. Similarly, variants in *IGF2BP2* have been associated with reduced beta cell function [5] and reduced insulin secretion [41], but further investigation is needed to understand how this variant has a greater effect on the progression from IFG to overt diabetes than on that from NFG to IFG.

Conclusions We have found that individuals carrying known type 2 diabetes risk loci at *GCK*, *SLC30A8* and *MTNR1B* progressed at a greater rate from NFG to IFG than from IFG to type 2 diabetes over a 7 year period of observation and were more likely to be classified as having IFG than NFG at the baseline observation. Over the same period of time, individuals carrying the risk allele at *IGF2BP2* progressed at a greater rate from IFG to type 2 diabetes than from NFG to IFG and were more likely to be classified as type 2 diabetic than as having IFG at the baseline observation. The biology by which these loci exert different degrees of influence on these clinical transitions remains speculative and needs to be explored further. These findings, if confirmed, hold implications for a better understanding of the genetic contribution to the development of IFG and type 2 diabetes.

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Contribution statement JCF, GAW and TI were involved with conception and design. JCF, GAW, TG, BN, MJD, JIR, SFAG, CSF, JSP, JGW, JBM, DSS, and DWB were involved with analysis and interpretation of data. GAW, TG, BN, MJD and JCF were involved in drafting the article. GAW, TG, BN, TI, JIR, SFAG, CSF, JSP, JGW, JBM, DSS, DWB, MJD and JCF were involved with critical revisions for important intellectual content. GAW, TG, BN, TI, JIR, SFAG, CSF, JSP, JGW, JBM, DSS, DWB, MJD and JCF gave final approval of the version to be published.

Duality of interest J.B. Meigs currently has research grants from GlaxoSmithKline and serves on a consultancy board for Interleukin Genetics. J.C. Florez has received consulting honoraria from Daiichi-Sankyo and AstraZeneca. The remaining authors declare that there is no duality of interest associated with this manuscript.

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