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Research Paper

Glucose and Insulin-Related Traits, Type 2 Diabetes and Risk of Schizophrenia: A Mendelian Randomization Study

Zhiqiang Li [a,b,c,d,*], Peng Chen [e,f,g], Jianhua Chen [b,c,h], Yifeng Xu [h], Qingzhong Wang [b], Xingwang Li [b], Changgui Li [i], Lin He [b,d], Yongyong Shi [a,b,c,d,h,**]

[a] The Affiliated Hospital of Qingdao University, The Biomedical Sciences Institute of Qingdao University (Qingdao Branch of SJTU Bio-X Institutes), Qingdao University, No. 16 Jiangsu Road, Qingdao 266003, PR China
[b] Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), the Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, No. 1954 Huashan Road, Shanghai 200030, PR China
[c] Institute of Social Cognitive and Behavioral Sciences, Shanghai Jiao Tong University, No. 800 Dongchuan Road, Shanghai 200240, PR China
[d] Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, No. 1954 Huashan Road, Shanghai 200030, PR China
[e] Key Laboratory of Pathobiology, Ministry of Education, Jilin University, No. 45 Chaoyang Xi Road, Changchun 130021, PR China
[f] College of Basic Medical Sciences, Jilin University, No. 126 Xinmin Street, Changchun 130021, PR China
[g] National Institute of Digestive, Diabetes and Kidney Diseases, National Institutes of Health, 445 N 5th St, Phoenix, AZ 85004, USA
[h] Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, No. 600 Wanping Nan Road, Shanghai 200030, PR China
[i] Shandong Provincial Key Laboratory of Metabolic Disease, The Metabolic Disease Institute of Qingdao University, No. 16 Jiangsu Road, Qingdao 266003, PR China

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ABSTRACT

Background: The link between schizophrenia and diabetes mellitus is well established by observational studies; however, the cause-effect relationship remains unclear.
Methods: Here, we conducted Mendelian randomization analyses to assess a causal relationship of the genetic variants related to elevated fasting glucose levels, hemoglobin A_{1c} (HbA_{1c}), fasting insulin levels, and type 2 diabetes with the risk of schizophrenia. The analyses were performed using summary statistics obtained for the variants identified from the genome-wide association meta-analyses of fasting glucose levels (up to 133,010 individuals), HbA_{1c} (up to 153,377 individuals), fasting insulin levels (up to 108,557 individuals), type 2 diabetes (up to 659,316 individuals), and schizophrenia (up to 108,341 individuals). The association between each variant and schizophrenia was weighted by its association with each studied condition, and estimates were combined using an inverse-variance weighted meta-analysis.
Findings: Using information from thirteen variants related to fasting insulin levels, the causal effect of fasting insulin levels increases (per 1-SD) on the risk of schizophrenia was estimated at an odds ratio (OR) of 2·33 ($p = 0{\cdot}001$), which is consistent with findings from the observational studies. The fasting glucose associated single nucleotide polymorphisms (SNPs) had no effect on the risk of schizophrenia in Europeans and East Asians ($p > 0{\cdot}05$). Nonsignificant effects on the risk of schizophrenia was observed with raised HbA_{1c} and type 2 diabetes, and consistent estimates were obtained across different populations.
Interpretation: Our results suggest a causal role of elevated fasting insulin levels in schizophrenia pathogenesis.

1. Introduction

Schizophrenia is a chronic and severe disorder that affects approximately 1% of the general population [1]. The causes and pathogenesis of schizophrenia remain largely unclear, with various factors proposed and others discounted or modified [2]. Patients with schizophrenia are more likely to develop impaired glucose tolerance, insulin resistance, and type 2 diabetes [3, 4]. Antipsychotic medication is considered a key factor associated with the prevalence of these abnormalities [3, 5]. However, antipsychotic-naïve, first-episode schizophrenia patients were also found to have significantly impaired glucose tolerance and

* Correspondence to: Z. Li, The Affiliated Hospital of Qingdao University, The Biomedical Sciences Institute of Qingdao University (Qingdao Branch of SJTU Bio-X Institutes), Qingdao University, No. 16 Jiangsu Road, Qingdao 266003, PR China.
** Correspondence to: Y. Shi, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), The Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, No. 1954 Huashan Road, Shanghai 200030, PR China.
E-mail addresses: lizqsjtu@gmail.com (Z. Li), shiyongyong@gmail.com (Y. Shi).

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Research in Context

Evidence before This Study

Observational studies suggest that impaired glucose homeostasis is associated with schizophrenia (even for antipsychotic-naïve, first-episode schizophrenia). However, knowledge of the mechanisms underlying the association is limited. Findings from the observational studies might be influenced by complex confounders, which could be better controlled by using genetic markers as instruments. Linkage disequilibrium score regression and Mendelian randomization analyses were used to test the relationships between schizophrenia and impaired glucose homeostasis using genetic information, and no causal relationship was observed. However, some potential confounders (such as genetic differences among populations) were omitted, some samples were small (such as the fasting insulin levels analysis), and these factors might have influenced the findings.

Added Value of This Study

We performed a Mendelian randomization study to determine the causality between impaired glucose homeostasis-related traits and schizophrenia based on the effect size estimates derived from data sets with a large sample size (fasting glucose levels, up to 133,010 individuals; HbA1c, up to 153,377 individuals; fasting insulin levels, up to 108,557 individuals; type 2 diabetes, up to 659,316 individuals; schizophrenia, up to 108,341 individuals), which increases the precision of causal inferences. Our analyses suggest that a genetic predisposition to higher fasting insulin levels is associated with an increased risk of schizophrenia. No causal relationship was observed between schizophrenia and fasting glucose levels, HbA_{1c} or type 2 diabetes.

Implications of all the Available Evidence

Our findings suggest a role for insulin in the potential pathophysiology of schizophrenia and indicate that the association of schizophrenia and type 2 diabetes is mainly driven by the pleiotropic actions of insulin. Further studies are needed to clarify the mechanisms of action, which might lead to novel therapies for treating schizophrenia or even for diabetes.

increased insulin levels compared with healthy controls [6], suggesting these metabolic dysregulations might be intrinsic to the pathogenesis of schizophrenia.

Though elevated fasting glucose levels, elevated fasting insulin levels, reduced glucose tolerance, and increased insulin resistance have been associated with schizophrenia in observational studies [7], whether these associations are causal remains unclear. A Mendelian randomization analysis may help to clarify the relationship [8]. This approach uses the genetic variants as instruments for making causal inferences and leverages the random assortment of alleles at the time of conception to overcome limitations inherent in observational studies, thus improving causal inferences.

In this study, a Mendelian randomization approach was used to determine the causality between hyperglycemia and hyperinsulinemia-related traits (fasting glucose levels, hemoglobin A_{1c} (HbA_{1c}), fasting insulin levels and type 2 diabetes) and schizophrenia.

2. Methods

2.1. Study Design and Data Sources

The Mendelian randomization approach uses known genetic variants associated with an exposure to estimate the causal relationship between this exposure and an outcome of interest. This study involved analyses of several of the most up-to-date publicly available and published data sets (Table 1).

Effect size estimates for single-nucleotide polymorphisms (SNPs) associated with glycemic and insulin traits (fasting glucose levels, HbA_{1c} and fasting insulin levels) were obtained from the MAGIC (Meta-Analyses of Glucose and Insulin-Related Traits Consortium) data sets of Europeans [9–11] and transethnic groups [11], and other reports of East Asians [12–15]. For the loci influencing fasting glucose levels in Europeans, the results were mainly from the MAGIC Metabochip Public data set (a data set of 133,010 European nondiabetic participants) [10]. For the loci influencing fasting glucose levels in East Asians, effect estimates were mainly obtained from the GWAS and replication meta-analyses of 46,085 individuals [13]. For the loci influencing HbA_{1c}, the results were mainly from analyses of ancestrally diverse populations (Europeans: 123,665 nondiabetic individuals; East Asians: 20,838 nondiabetic individuals and 42,790 Japanese individuals [15]; transethnic: 153,377 nondiabetic individuals) [11]. For the loci influencing fasting insulin levels, the results were from the MAGIC Metabochip Public data set of 108,557 European nondiabetic participants [10].

Effect size estimates for SNPs associated with type 2 diabetes were obtained from previously published European, Asian and trans-ancestry GWAS and replication analyses [16–23], most of which were from the DIAGRAM (Diabetes Genetics Replication and Meta-Analysis) Consortium or integrated data from the DIAGRAM Consortium. The results were mainly from the DIAGRAM data sets comprising either 41,221 cases and 171,526 controls [21] or 62,892 cases and 596,424 controls [24] for Europeans; a Japanese population-based analysis of 23,399 cases and 31,722 controls [18]; an AGEN (Asian Genetic Epidemiology Network) data set of 25,079 cases and 29,611 controls [16] for East Asians; and the trans-ethnic meta-analyses of 47,979 cases and 139,611 controls [19] or 73,337 cases and 192,341 controls [23].

Genetic data of the schizophrenia results from the Psychiatry Genomics Consortium (PGC2) [25] were obtained and comprised 49 samples of European ancestry (33,640 cases and 43,456 controls, EUR49) and 3 samples of East Asian ancestry (1836 cases and 3383 controls). Effect estimates were obtained from both the PGC2 and EUR49 data sets. Effect size estimates for Chinese schizophrenia GWAS analysis [26] (7711 cases and 18,327 controls, BIOX) and a trans-ethnic meta-analysis with PGC2 (43,187 cases and 65,166 controls in total, PGC2 + BIOX) [26] were also used.

Genome-wide summary statistics for body mass index (BMI) in a meta-analysis of about 700,000 European individuals [27] were obtained for conditional analyses.

Ethical review and informed consent had been obtained in all of the original studies.

2.2. SNP Selection

The previously identified genome-wide significantly ($p < 5 \times 10^{-8}$) associated SNPs for fasting glucose levels, HbA_{1c}, fasting insulin levels and type 2 diabetes were selected. For the East Asian analysis, the selected SNPs are from studies performed on East Asians (mainly included but not limited to Chinese and Japanese populations). For the European analysis, the selected SNPs are from studies of European descent. For the trans-ancestry analysis, the selected SNPs are from studies of multiancestry, in which the majority of the participants were of European and Asian descent. For each trait, the independent SNPs ($r^2 < 0{\cdot}01$ and window size = 2 Mb) were selected using the clumping function in PLINK [28]. The 1000 Genomes Project Phase 3 European

Table 1
The main data sets used in this study.

Trait/Disease	Publication[a]	Population(s)	Sample size	Source[b]
Fasting glucose levels	22885924	Europeans	133,010 non-diabetic individuals	MAGIC
	25187374	East Asians	46,085 individuals	AGEN
HbA_{1c}	28898252	Europeans	123,665 non-diabetic individuals	MAGIC
	28898252	East Asians	20,838 non-diabetic individuals	MAGIC
	29403010	Japanese	42,790 individuals	JENGER
	28898252	Trans-ethnic (Europeans, East Asians and South Asians)	153,377 non-diabetic individuals	MAGIC
Fasting insulin levels	22885924	Europeans	108,557 non-diabetic individuals	MAGIC
Type 2 diabetes	28566273	Europeans	41,221 cases and 171,526 controls	DIAGRAM
	30054458	Europeans	62,892 cases and 596,424 controls	DIAGRAM + GERA + UKB
	26818947	Japanese	23,399 cases and 31,722 controls	RIKEN Yokohama Institute
	22158537	East Asians	25,079 cases and 29,611 controls	AGEN
	24509480	Europeans, East Asians, South Asians, Mexicans, Mexican Americans	47,979 cases and 139,611 controls	DIAGRAM trans-ancestry analysis
	28869590	Europeans, East Asians, South Asians	73,337 cases and 192,341 controls	UPENN + DIAGRAM
Schizophrenia	25056061	Europeans	33,640 cases and 43,456 controls	PGC2-EUR49
	28991256	Chinese	7,699 cases and 18,327 controls	BIOX
	28991256	Europeans, East Asians	43,175 cases and 65,166 controls	PGC2 + BIOX

[a] PubMed ID.
[b] MAGIC, the Meta-Analyses of Glucose and Insulin-related traits Consortium; AGEN, the Asian Genetic Epidemiology Network; JENGER, Japanese ENcyclopedia of GEnetic associations by Riken; DIAGRAM, the DIAbetes Genetics Replication And Meta-analysis consortium; GERA, Genetic Epidemiology Research on Aging; UKB, the UK Biobank; UPENN, the University of Pennsylvania; PGC2-EUR49, the Psychiatric Genomics Consortium, 49 European samples; BIOX, Bio-X Institutes. HbA_{1c} indicates hemoglobin A_{1c}.

and/or East Asian datasets [29] were used to calculate linkage disequilibrium between the variants.

2.3. Statistical Analysis

We tested fasting glucose levels, HbA_{1c}, fasting insulin levels and type 2 diabetes separately for the causality of schizophrenia. To assess for violations of the Mendelian randomization assumptions, Cochran's Q and MR-Egger tests were used to detect heterogeneity and directional pleiotropy of the genetic instruments [30, 31]. We also used an MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) for pleiotropy and outlier SNPs detection [32]. The inverse-variance weighted (IVW) method [33] was adopted to combine SNP-specific causal estimates for schizophrenia after exclusion of the outlier SNPs. We also produced scatter, funnel, forest and leave-one-out plots for further assessment and interpretation. The difference between the magnitudes of the causal estimates from different populations was evaluated using a z-score based test. All statistical analyses were performed using R (version 3.4.0, R Foundation) and the related packages (TwoSampleMR, MR-PRESSO and MendelianRandomization) [34]. Conditional analyses were performed using mtCOJO in the GCTA software package to account for major confounders (e.g. BMI) [35]. The adjusted estimates from the mtCOJO analysis were then used in the Mendelian randomization analysis to give a conditional effect. The threshold of statistical significance for the MR IVW analyses (main outcome measures) was $p < 0{\cdot}005$ ($0{\cdot}05/10$, Bonferroni-corrected for 10 tests: two for fasting glucose levels, three for HbA1c, two for fasting insulin levels and three for type 2 diabetes). The threshold of significance for other analyses, such as the heterogeneity and pleiotropy analyses (quality control measures), was $p < 0{\cdot}05$.

3. Results

3.1. Fasting Glucose Levels, HbA_{1c} and Schizophrenia

Forty loci were associated with fasting glucose levels in Europeans and/or East Asians. To investigate the consistency and directional effect of the SNP association with fasting glucose levels and schizophrenia, we plotted the effect and standard error of SNPs on fasting glucose levels with their corresponding effect and standard error on the risk of schizophrenia for each data set. We observed substantial heterogeneity in the effect estimates for the 33 independent fasting glucose associated SNPs (Table S1) in Europeans (heterogeneity $p = 2{\cdot}68 \times 10^{-5}$). The MR-PRESSO test also showed outlier pleiotropy and suggested three SNP outliers. After excluding outlier SNPs (Table S1), heterogeneity and pleiotropy were eliminated (heterogeneity $p = 0{\cdot}273$ and MR-PRESSO $p = 0{\cdot}304$). The MR-Egger intercept test also provided no evidence against the null hypothesis of no unmeasured pleiotropy for the final instrument variable set (intercept $p = 0{\cdot}924$). We then sought to estimate the causal effects of fasting glucose levels on the risk of schizophrenia using the Mendelian randomization. The 30 fasting glucose associated SNPs in Europeans had a nominally significant effect on the risk of schizophrenia (IVW estimate of odds ratio, OR: 0·84, 95% Confidence Interval (CI): 0·71–0·99, $p = 0{\cdot}038$; Fig. 1 and Fig. S1 in the appendix), as it did not pass Bonferroni correction. For the 14 independent SNPs associated with fasting glucose levels in East Asians (Table S2), no evidence of heterogeneity or pleiotropy was observed from any of the Cochran's Q ($p = 0{\cdot}548$), MR-PRESSO ($p = 0{\cdot}591$) or MR-Egger (intercept $p = 0{\cdot}645$) tests. Using these genetic variants as instrumental variables, we found that the evaluated fasting glucose levels had no significant effect on the risk of schizophrenia in East Asians (IVW OR: 1·04, 95% CI: 0·84–1·27, $p = 0{\cdot}737$; Fig. 1 and Fig. S2 in the appendix). There were also no significant differences between the estimated effects of fasting glucose levels on schizophrenia derived from European and East Asian analyses (p for difference > 0·05, Fig. S3 in the appendix).

Cochran's Q and MR-PRESSO tests showed evidence of heterogeneity ($p = 3{\cdot}30 \times 10^{-3}$) and outlier pleiotropy ($p = 4{\cdot}50 \times 10^{-3}$) in the effect estimates for the 38 independent HbA_{1c}-associated SNPs in Europeans (Table S3). After excluding two outlier SNPs identified by MR-PRESSO (Table S3), we formed the final instrument variable set with no evidence of heterogeneity and pleiotropy ($p > 0{\cdot}05$ for all the Cochran's Q, MR-PRESSO and MR-Egger analyses). The instrument set had a nonsignificant effect on the risk of schizophrenia (IVW OR: 0·94, 95% CI: 0·72–1·21, $p = 0{\cdot}612$, Fig. 1 and Fig. S4 in the appendix). For the 27 independent HbA_{1c}-associated SNPs in East Asians (Table S4), no evidence of heterogeneity and pleiotropy was observed ($p > 0{\cdot}05$ for all the related analyses) and the effect on the risk of schizophrenia was also nonsignificant (IVW OR: 0·88, 95% CI: 0·70–1·12, $p = 0{\cdot}301$, Fig. 1 and Fig. S5 in the appendix). By removing the outlier from the 47 independent HbA_{1c}-associated SNPs for the trans-ethnic analysis (Table S5), the instrument set also showed a nonsignificant effect on the risk of schizophrenia (IVW OR: 0·92, 95% CI: 0·73–1·17, $p = .500$, Fig. 1 and

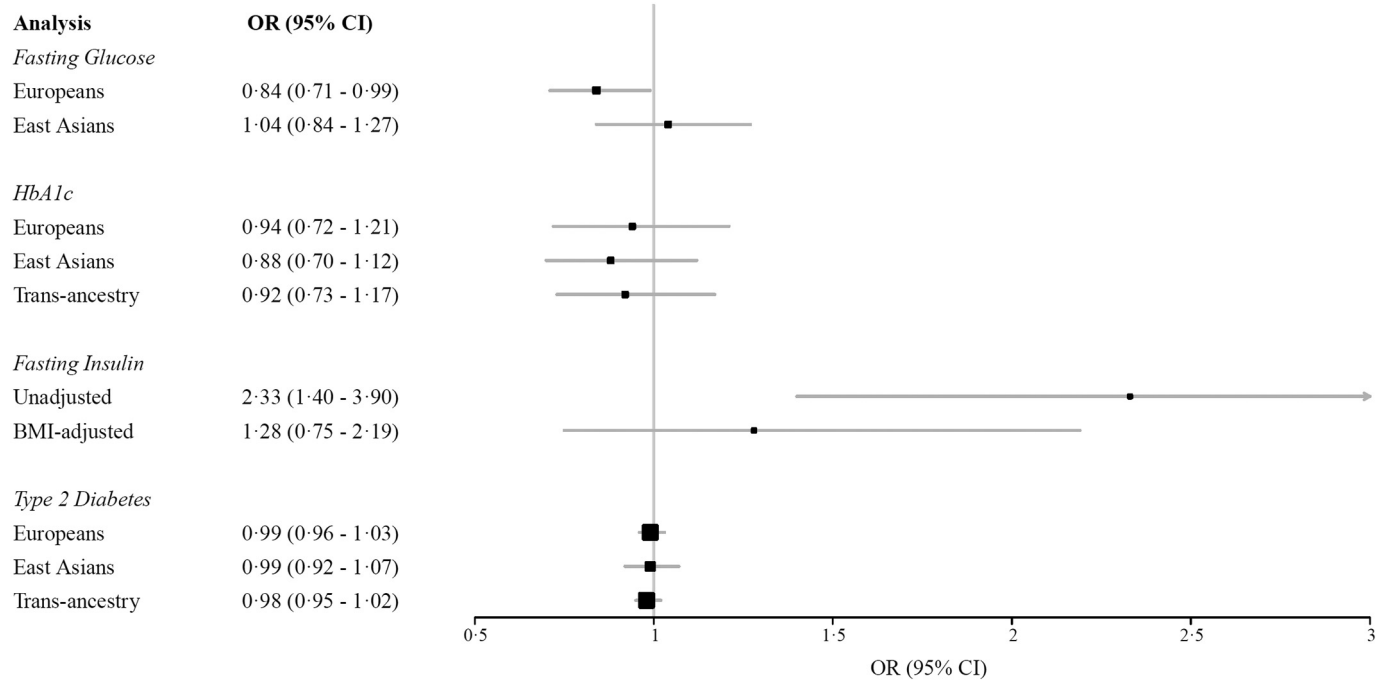

Fig. 1. Mendelian Randomization Estimated Effects of Glucose and Insulin-Related Traits, Type 2 Diabetes on Schizophrenia. OR, odds ratio; 95% CI, 95% confidence interval.

Fig. S6 in the appendix). No evidence for the presence of heterogeneity and pleiotropy was observed for the related analyses (all $p > 0{\cdot}05$). No differences were observed for the causal effect estimates from different populations (p for difference $> 0{\cdot}05$ for all comparisons, Fig. S7 in the appendix).

3.2. Fasting Insulin Levels and Schizophrenia

Nineteen independent loci were found to be associated with fasting insulin levels and/or BMI-adjusted fasting insulin levels. Considering the complicated relationship between insulin secretion and weight gain [36, 37], we performed separate analyses for the unadjusted and BMI-adjusted estimates. For the 14 independent SNPs associated with fasting insulin levels (Table S6), we observed some evidence of heterogeneity and outlier pleiotropy in the effect estimates (heterogeneity $p = 0{\cdot}018$ and MR-PRESSO $p = 0{\cdot}023$). Although the MR-PRESSO test did not indicate any significant outlier SNPs, we noted that the association for one (the *FTO* locus) of the 14 SNPs was totally abolished by BMI [10], which is most likely the potential source of heterogeneity and pleiotropy. We thus excluded the variant from the analysis according to the common practice [10, 38]. The heterogeneity and outlier pleiotropy was eliminated ($p > 0{\cdot}05$) when restricting the analysis to the 13 SNPs (Table S6). The MR-Egger test also indicated no directional pleiotropic bias ($p > 0{\cdot}05$). Under the IVW model, the genetic variants associated with fasting insulin levels presented an OR of 2·33 (95% CI: 1·40–3·90, $p = 0{\cdot}001$, Fig. 1 and Fig. S8 in the appendix) per 1-SD fasting insulin levels increase in the risk of schizophrenia. For the BMI-adjusted analysis (Table S7), evidence for the presence of heterogeneity ($p = 5{\cdot}05 \times 10^{-4}$) and outlier pleiotropy ($p = 5{\cdot}00 \times 10^{-4}$) were observed. Removal of the outlier SNPs eliminated the evidence ($p > 0{\cdot}05$; Table S7). We observed a nonsignificant effect (IVW OR: 1·28, 95% CI: 0·75–2·19, $p = 0{\cdot}369$; Fig. 1 and Fig. S9 in the appendix) for the final instrument set. The MR-Egger test also indicated no evidence for unmeasured directional pleiotropy ($p > 0{\cdot}05$). No differences were observed for the causal effect estimates between these two analyses (p for difference $> 0{\cdot}05$, Fig. S10 in the appendix).

Since a significant effect of elevated fasting insulin levels causing schizophrenia was observed, we also performed the reverse Mendelian randomization analysis aimed to investigate the causal effect of schizophrenia on fasting insulin levels and observed no evidence that schizophrenia contributes to evaluated fasting insulin levels (IVW OR: 1·00, 95% CI: 0·99–1·01, $p = 0{\cdot}969$, Fig. S11 in the appendix). The Cochran's Q, MR-PRESSO and MR-Egger tests indicated no evidence for heterogeneity or pleiotropy of the genetic instrumental markers (all $p > 0{\cdot}05$), suggesting that the IVW regression returns unbiased estimates for the causal effect.

3.3. Type 2 Diabetes and Schizophrenia

More than 140 susceptibility loci have been identified for type 2 diabetes in at least one of the GWAS analyses for Europeans, East Asians and trans-ancestry groups. The Cochran's Q and MR-PRESSO tests showed heterogeneity and outlier pleiotropy in the European and trans-ancestry analyses. After excluding outlier SNPs, 120 independent type 2 diabetes-associated SNPs were used as the genetic instruments for the Mendelian randomization analyses for Europeans (Table S8), 37 independent SNPs were used for East Asians (Table S9), and 59 independent SNPs were used for trans-ancestry (Table S10). We did not observe a causal role of type 2 diabetes for schizophrenia in any of these analyses (IVW OR: 0·99, 0·99, and 0·98, $p = 0{\cdot}616$, 0·829, and 0·351, Fig. 1 and Figs. S12, S13, and S14 in the appendix). None of the intercept estimates from the MR-Egger method were significantly deviated from zero ($p = 0{\cdot}861$, 0·257, and 0·261). No evidence of heterogeneity and outlier pleiotropy was observed ($p > 0{\cdot}05$) for all tests. The estimated effects of type 2 diabetes on schizophrenia in different populations were consistent (p for difference $> 0{\cdot}05$, Fig. S15 in the appendix).

3.4. Estimates Conditional on BMI

It is important to remove potential confounders in a Mendelian randomization analysis [35, 39], we thus tried to investigate whether BMI (a major confounder) bias the Mendelian randomization estimates using the mtCOJO analysis [35]. Because it required genome-wide summary statistics for both of confounder and exposure, the conditional analyses were conducted to assess the effect sizes conditional on BMI only for HbA_{1c} and type 2 diabetes in Europeans. Both these analyses remained nonsignificant, and the estimates were consistent with

those from the unconditional analyses. The conditional effect on schizophrenia is 0·94 and 0·98 for HbA_{1c} and type 2 diabetes, respectively.

4. Discussion

We performed a Mendelian randomization analysis for causal inference using genetic instrumental variables from known associations for glucose and insulin-related traits and type 2 diabetes on schizophrenia. Our analyses suggested that a genetic predisposition to higher fasting insulin levels was causally linked to an increased risk of schizophrenia, whereas the schizophrenia-associated variants had a nonsignificant effect on fasting insulin levels. The asymmetry in the effects of the genetic variants on schizophrenia and fasting insulin levels supports the fact that fasting insulin levels (the 'causal' trait) is one of the causal factors that influences schizophrenia (the 'caused' disease) [40]. The effect magnitude for BMI-adjusted fasting insulin levels was smaller than the unadjusted one. Nonsignificant effects on the risk of schizophrenia were observed for the genetic variants associated with fasting glucose levels, HbA_{1c} and type 2 diabetes.

Recently, Toby Pillinger et al. [7] reported that individuals with first-episode schizophrenia had significantly elevated levels of fasting glucose (*Hedges' g*: 0·20, 95% CI: 0·02–0·38) and fasting insulin (*Hedges' g*: 0·41, 95% CI: 0·09 to 0·72), as well as elevated glucose levels after an oral glucose tolerance test and greater insulin resistance compared with healthy controls. However, no significant differences were demonstrated in HbA_{1c} levels (*Hedges' g*: −0·08, 95% CI: −0·34–0·18). The estimate (OR effect size converted from *Hedges' g*) [41] for the evaluated fasting insulin levels was consistent with our causal effect estimates derived from the Mendelian randomization (Fig. S10 in the appendix). Furthermore, consistent results were also observed for HbA_{1c} levels, with a nonsignificant effect on the risk of schizophrenia (Fig. S7 in the appendix). However, inconsistent findings were observed for fasting glucose levels; our analyses did not support a causal role of elevated fasting glucose levels and the estimate from our Mendelian randomization analyses in Europeans showed the opposite direction compared to the literature estimate (Fig. S3 in the appendix).

Given that type 2 diabetes generally occurs in middle-aged adults (after age 45) while schizophrenia usually strikes in young adulthood (before age 30) [7], and antipsychotic treatment may increase the risk for diabetes [42], it is infeasible to assess the effects of type 2 diabetes on the risk of schizophrenia by observational studies. The observational studies usually evaluate the prevalence of diabetes mellitus in patients suffering from schizophrenia [3, 42, 43]. Davy Vancampfort et al. reported that the relative risk of type 2 diabetes among patients with schizophrenia or related psychosis when compared with healthy controls is 2·04 (95% CI: 1·69–2·49) [43]. However, our Mendelian randomization analyses did not support the causal role of type 2 diabetes in the risk of schizophrenia and gave estimates of a nonsignificant reduction in risk.

To our knowledge, our results provide the first significant evidence for supporting a causal role of elevated fasting insulin levels on the risk of schizophrenia. Insulin is a hormone made and secreted by the pancreas, a gland behind the stomach. Insulin plays a major role in the regulation of energy metabolism, not only in the body but also in the brain [44]. Insulin is known as a neuropeptide and plays a key role in neurotropism, neuromodulation and neuroplasticity [45]. In our recent pathway enrichment analysis for schizophrenia using the largest trans-ancestry GWAS data set (108,341 individuals) [26], the top two most significant schizophrenia-associated pathways are the regulation of insulin secretion of glucagon-like peptide 1 and the inhibition of insulin secretion by adrenaline/noradrenaline (p for enrichment $= 5{\cdot}14 \times 10^{-7}$ and $3{\cdot}81 \times 10^{-5}$, respectively) [26].

No causal effects of the fasting glucose levels, HbA_{1c} and type 2 diabetes on schizophrenia were obtained in our genetic analyses from the different populations. Especially for the effect of type 2 diabetes on schizophrenia, our analyses for Europeans, East Asians and trans-ancestry gave consistent estimates of 0·98 to 0·99, which are in the opposite direction of estimates from the observational studies [42, 43]. Similarly, using a linkage disequilibrium score regression-based analysis of genome-wide results, Bulik-Sullivan B et al. found a nonsignificant genetic correlation ($rg = -0{\cdot}028$, $p = 0{\cdot}618$) between type 2 diabetes and schizophrenia in Europeans [46]. In addition, Pickrell JK et al. identified one notable genomic region where a variant influenced both type 2 diabetes and schizophrenia, and the genetic variant had an opposite effect on the risk of them [40]. Given that a causal role was only observed for a genetic predisposition to higher fasting insulin levels and increased risk of schizophrenia and not for type 2 diabetes and its key symptoms (raised fasting glucose levels and HbA1c), we speculate that the mechanisms, whereby elevated fasting insulin levels may increase the risk of schizophrenia, are likely to take effect via other pathways rather than diabetes-related insulin signaling pathways. Schizophrenia might be a consequence of disturbances in insulin, probably via a direct effect on brain function, but not a consequence of impaired glucose metabolism. However, further work will be required to investigate the potential mechanisms and elucidate the translational implications.

These Mendelian randomization analyses had several strengths. First, by using randomly allocated variants as instrumental variables, we were able to reduce the potential effects of confounding factors and reverse causation observed in observational studies. In addition, the differences observed from the genetic variants could represent the lifelong effects of some conditions (such as HbA_{1c} and type 2 diabetes). Second, the estimates we used were derived from studies of large sample sizes (schizophrenia, up to 108,341 individuals; fasting glucose levels, up to 133,010 individuals; HbA_{1c}, up to 153,377 individuals; fasting insulin levels, up to 108,557 individuals; type 2 diabetes, up to 659,316 individuals), which increases the precision of the estimates. In contrast to a recent study of Mendelian randomization analysis [47], where schizophrenia was not causally related to impaired glucose homeostasis, our study integrated results from the most up-to-date publications, and included more newly identified trait-associated SNPs. Third, while it is assumed that the effects across populations are consistent in the trans-ethnic meta-analysis, genetic heterogeneity has often been observed [48]. Due to differing causal variants or linkage disequilibrium patterns in the populations of different ancestries [49], the conditions associated with the variants might be different or have varied estimates in different populations, and these may have had some influence on the results. If applicable, we performed these analyses separately for each population to minimize these potential effects. These factors were not sufficiently taken into account in the previous Mendelian randomization analysis [47]. Finally, several methods were used to detect and correct for a possible pleiotropic bias (MR-Egger, Cochran's Q, MR-PRESSO and exclusion of outlier pleiotropic SNPs).

There were also some limitations in our study. First, due to a lack of genetic data, we were able to perform all of the European, East Asian and trans-ethnic analyses for type 2 diabetes and HbA1c only. The trans-ethnic analysis lacked the other variants and the East Asian analysis also lacked the fasting insulin levels. Moreover, the sample size was relatively small in the East Asian studies compared to the European ones. Thus, the number of the associated variants for analysis was always lower than that in the Europeans. Third, due to the lack of a genetic study with an adequate sample size, we were limited in our ability to evaluate associations with homeostatic model assessment indices. Analysis of the direct estimates for β-cell function, insulin sensitivity and insulin secretion might have been helpful for unravelling the association between schizophrenia and impaired glucose homeostasis more clearly.

In summary, our findings provide evidence in support of the causal role of elevated fasting insulin levels but not fasting glucose levels, HbA_{1c} and type 2 diabetes in schizophrenia.

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Declaration of Interests

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Author Contributions

ZQL, and YYS designed and conceived the study. ZQL, PC, JHC, YFX, QZW, XWL, CGL, and LH contributed to data acquisition. ZQL, and YYS supervised data entry and integrity. ZQL analysed data and prepared the figures. ZQL, YYS, and PC wrote the manuscript. All authors reviewed and approved the final draft.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.07.037.

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