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Additional common variants associated with type 2 diabetes and coronary artery disease detected using a pleiotropic cFDR method

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Abstract

Genome-wide association studies (GWASs) have been performed extensively in diverse populations to identify single nucleotide polymorphisms (SNPs) associated with complex diseases or traits. However, to date, the SNPs identified fail to explain a large proportion of the variance of the traits/diseases. GWASs on type 2 diabetes (T2D) and coronary artery disease (CARD) are generally performed as single-trait studies, rather than analyzing the related traits simultaneously. Despite the extensive evidence suggesting that these two phenotypes share both genetic and environmental risk factors, the shared overlapping genetic biological mechanisms between these traits remain largely unexplored. Here, we adopted a recently developed genetic pleiotropic conditional false discovery rate (cFDR) approach to discover novel loci associated with T2D and CARD by incorporating the summary statistics from existing GWASs of these two traits. Applying the cFDR level of 0.05, 33 loci were identified for T2D and 34 loci for CARD, 9 of which for both. By incorporating pleiotropic effects into a conditional analysis framework, we observed that there is significant pleiotropic enrichment between T2D and CARD. These findings may provide novel insights into the etiology of T2D and CARD, as well as the processes that may influence disease development both individually and jointly.

Keywords

T2D; Type 2 diabetes; Coronary artery disease; Pleiotropic; Conditional FDR

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Author contributions statement

Qiang Zhang as the first author performed data analysis and wrote the manuscript. Wan Qiang Lv, Hui Min Liu, Jing Yang He and Xin Xia provided advice and suggestions while we met some problems during the data analysis process. Wei Dong Zhang and H.W.D gave constructive suggestions during the whole process. Chang Qing Sun conceived and initiated this project, provided advice on experimental design, oversaw the implementation of the statistical method, and revised/finalized the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2018.09.003.

1. Introduction

Genome-wide association studies (GWASs) have successfully identified hundreds of SNPs associated with complex diseases or traits. However, the SNPs identified to date fail to explain a large proportion of the variance and risks involved. Previous studies have suggested that GWAS has the potential to explain a larger proportion of this "missing heritability"[1,2] mainly by using enlarged sample sizes.[3] However, although acquiring larger sample sizes may increase statistical power, it is often not feasible since the recruiting and genotyping of additional participants is too costly. Therefore, there is a need for analytical methods that can better and more efficiently utilize the information contained in the existing pool of available data for the identification of trait-associated loci. Several of these types of methods have recently been developed[4–6] and successfully applied[7,8] to identify novel loci for various complex traits.

Pleiotropy is the phenomenon of a single gene affecting two or more phenotypes.[9] There is ample evidence to suggest that genetic pleiotropy exists in many correlated diseases and traits, such as bipolar disorder and schizophrenia,[10] indicating that related traits may share overlapping genetic mechanisms. Through the incorporation of information regarding genetic pleiotropy, we can improve the detection power of common variants associated with complex diseases or traits by effectively increasing the sample sizes without the need to recruit more individuals. The joint analysis of related phenotypes may reveal novel insights into the common biological mechanisms and overlapping pathophysiological relationships between complex traits.

Andreassen et al.[4] developed a genetic-pleiotropy-informed conditional false discovery rate (cFDR) method by leveraging two independent GWASs from associated traits in a conditional analysis. The method has been successfully applied to genetically associated diseases and phenotypes including schizophrenia and bipolar disorder,[7] as well as blood pressure and other phenotypes.[8] Our group has recently successfully applied the cFDR method to the joint analyses of bone mineral density (BMD) and breast cancer,[11] BMD and coronary artery disease (CARD),[12] femoral neck (FNK) BMD and height,[13] and CARD and birth weight.[14] All of these studies improved statistical power through the joint analysis of related traits, and unambiguously demonstrated the utility of the method for improving gene discovery in the identification of potentially novel trait-associated variants.

Type 2 diabetes (T2D) is a long term chronic metabolic disorder mainly characterized by high blood sugar, insulin resistance and relative lack of insulin. Long term exposure to high blood sugar will result multiple complex complications like stroke, diabetic retinopathy and heart disease.[15] Epidemiological studies estimate that 422 million people were living with diabetes, with a worldwide prevalence of 8.3% in 2014.[15] As the most common complication of T2D, cardiovascular disease is the most primary cause of T2D mortality and mobility.[16] The overall prevalence of CARD in diabetic adult individuals was reported as 55% and an estimated 75% of the T2D patients died of cardiovascular disease.[17] Heritability studies demonstrate a substantial genetic contribution to T2D risk (h^2~40–70%)[18] and CARD risk (h^2~30–60%).[19]

Multiple prospective studies suggested that diabetic individuals have 1.5 to threefold increased risk of developing coronary heart disease compared to the nondiabetic individuals.[20] What's more, compared with nondiabetic individuals, the mortality rate of cardiovascular disease is more than twice in men and more than fourfold in women who have diabetes.[21] There is strong evidence[21,22] that T2D and CARD share primary risk factors such as smoking, hypertension, elevated lipid, dysbetalipoproteinemia and hyperglycemia, also some potential risk factors like obesity, lack of physical activity, cardiovascular family history, gender and age. Although dozens of genetic loci associated with T2D or CARD have been demonstrated by GWASs, these loci can explain at best 10% of the genetic variance for either T2D[23] or CARD.[24] Considering the high degree of heritability, close relationship and potential pleiotropy between these two phenotypes, we assume those two traits are ideal for the further analyses using the cFDR approach to improve the detection of loci associated with T2D or CARD or both and explore their common etiology.

In this study, we applied the genetic-pleiotropy-informed cFDR method[4] on two large and independent GWAS summary statistics of T2D and CARD[23,24] to identify novel loci and pleiotropic relationships between T2D and CARD. The purpose of our study is to improve SNP detection for T2D and CARD with these two existent GWASs and gain some novel insights into shared biological mechanisms and overlapping genetic heritability between them.

2. Materials and methods

2.1. GWAS Datasets

The dataset for T2D contains association summary statistics of 12 GWASs of European descent which compromising of 12,171 cases and 56,862 controls.23 The dataset was downloaded from http://www.diagram-consortium.org/downloads.html. The meta-analyses were previously performed by the DIAbetes Genetics Replication And Metaanalysis (DIAGRAM) Consortium. The dataset for CARD contains association summary statistics of 22 GWASs of European descent which comprising of 22,233 cases and 64,762 controls.[24] The dataset was downloaded from http://www.cardiogramplusc4d.org/data-downloads. The dataset was conducted by the transatlantic Coronary ARtery Disease Genome-wide Replication and Meta-analysis (CARDloGRAM) Consortium. Both of the datasets consist of the summary statistics for each SNP, providing the p values that have undergone genomic control at the individual study level, and again after meta-analysis. Further details of the samples and methods employed within each group are presented in the corresponding consortium papers.[23,24] We further checked the original studies in both GWASs (Table S1), there was one common study between these two GWASs datasets, WTCCC (1926 cases of T2D, and 71.5% × (1926 + 2938) = 3478 cases of CVD[25]), which makes the rates of CVD in the T2D GWAS and the rates of diabetes in the CVD GWAS are 3% and 5% respectively.

The dataset for attention-deficit/hyperactivity disorder (ADHD) contains association summary statistics of European descent which compromising of 5415 individuals (2064 trios, 896 cases and 2455 controls),[26] the dataset was downloaded from https://www.med.unc.edu/pgc/results-and-downloads/data-use-agreement-forms/ADHD_data_download_agreement. The dataset for major depressive disorder (MDD)

contains association summary statistics of 18,759 independent and unrelated subjects of European ancestry (9240 MDD cases and 9519 controls),[27] The dataset was downloaded from https://www.med.unc.edu/pgc/results-and-downloads/data-use-agreement-forms/MDD_data_download_agreement. Both meta-analyses were previously performed by the Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium (PGC).

2.2. Conditional false discovery rate

The cFDR approach is well-established now and has been widely applied by many other groups[4,7,8,28,29] and our group.[12–14,30] We briefly summarize this cFDR approach as follows: after the data preparation processing as indicated in the previous papers, we computed the conditional empirical cumulative distribution functions (cdfs) of the corrected p-values for the x axis in conditional QQplot. Empirical cdfs for T2D SNP p-values were conditioned on nominal p-values in CARD, and *vice versa.* For each nominal p-value, an estimate of the cFDR was obtained from the conditional empirical cdfs. Using this cFDR approach, we obtained two cFDR tables–cFDR result for T2D conditioned on CARD and *vice versa*. Using these tables we identified loci associated with T2D and CARD (cFDR <0.05), respectively. Then a conjunction method was used to find SNPs significantly associated with both T2D and CARD. Specifically, we took the maximum of those two cFDR values above as our conjunction FDR.

2.3. Conditional QQ and enrichment plots for assessing pleiotropic enrichment

To assess the pleiotropic enrichment of SNP association compared to that expected under the null hypothesis, we presented conditional QQ plots based on different levels of significance of the conditional phenotype. The QQplots show the observed distribution of p-values plotted against the expected distribution of p-values under the null hypothesis. We plotted the QQ curve for the quantiles of nominal $-\log_{10}(p)$-values obtained from GWAS summary statistics for association of the subset of SNPs that are below each significance threshold in the conditional trait. The nominal $-\log_{10}(p)$-values are plotted on the y-axis and the empirical quantiles (cdfs) of the nominal p-values are plotted on the x-axis. Pleiotropic enrichment is expressed as the degree of leftward shift from the expected null line, and as the p values of the conditional phenotypes decrease, earlier leftward shift from the null line will persist.

In order to check the pleiotropic enrichment and provide a baseline that can be used to confirm novel findings, we also generated conditional QQ plots for two traits that are unlikely to be correlated with T2D and CARD, ADHD and MDD, as “control traits.”

2.4. Conditional Manhattan plots for localizing genetic variants

To demonstrate the localization of the SNPs associated with T2D conditional on their significance on CARD, and the reverse, we present conditional Manhattan plots. The plots present the relationship between all SNPs within an LD block and their chromosomal locations. The 22 chromosomal locations are plotted on the x-axis, and the $-\text{logi}_0(\text{FDR})$ T2D values conditional on CARD are plotted on the y-axis and *vice versa* for CARD. Any SNP with a $-\log_{10}(\text{FDR})$ value >1.3 (FDR < 0.05) was deemed to be significantly associated

with the principal phenotype. We also present a conjunction Manhattan plot to demonstrate the locations of the common pleiotropic genetic variants associated with both phenotypes.

2.5. Functional annotation and gene enrichment analysis

In order to evaluate the biological functions of the individual trait associated loci identified by cFDR and pleiotropic loci identified by conjunction FDR, we performed functional annotation and gene enrichment analysis using the gene ontology (GO) terms database (http://geneontology.org/.).[31] All significant genes identified by cFDR and conjunction FDR in our study were annotated and characterized based on three main categories: biological processes, cellular component and molecular functions. This analysis provided comprehensive biological information, allowing us to partially validate our findings by determining specific genes that are enriched in T2D-and CARD-related GO terms.

2.6. Protein-protein interaction network

In order to detect interactions and associations of the T2D-associated and CARD-associated genes respectively, protein-protein interaction analyses were conducted by searching the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (http://string-db.org/). The STRING database comprises known and predicted associations from curated databases or high-throughput experiments, and also with other associations derived from text mining, co-expression, and protein homology.[32]

3. Results

3.1. Assessment of pleiotropic enrichment

As an intuitive illustration, we present the data as conditional Q-Q plots (Fig. 1) to graphically assess the pleiotropic enrichment of SNPs of the principal phenotype successively conditioning on various strengths of associations with the conditional phenotype. Under the global null hypothesis, the theoretical distribution of p-values is expected to lie approximately on the diagonal line of the Q-Q plots. Enrichment of genetic associations is indicated as a leftward deflection from the null line as the principal phenotype is successively conditioned on increasing strength of associations with conditional phenotype. The degree of deflection between curves provides important information about the degree of pleiotropy between the two phenotypes. Larger deflection is considered as a greater enrichment of pleiotropic genes between the two phenotypes.

The conditional Q-Q plot for T2D conditional on CARD (A in Fig. 1) shows some enrichment across varying significance thresholds for CARD. The presence of leftward shift when restricting the analysis to include the SNPs that have more significant associations with T2D indicates an increase in the number of true associations for a given CARD p-value. Similar enrichment is observed for CARD given T2D (B in Fig. 1), as there appears to be a similar departure pattern between the different curves. These earlier deflections from the null line indicate a great proportion of true associations for any given T2D nominal p-value.

On the other hand, as negative controls, the conditional Q-Qplots for T2D given nominal p-values of association with ADHD (A in Fig. S1), CARD given nominal p-values of association with ADHD (C in Fig. S1), T2D given nominal p-values of association with MDD (A in Fig. S2), and CARD given nominal p-values of association with MDD (C in Fig. S2) all show no enrichment, and *vice versa.*

3.2. T2D loci identified with cFDR

Conditional on their association with CARD, we identified 33 significant SNPs (cFDR <0.05) for T2D variation (A in Fig. 2 and Table 1), which were mapped to 13 different chromosomes (1,3, 5–12,15–17) and annotated to 37 genes. In the original meta-analysis for T2D GWAS,[23] 16 SNPs had p-values smaller than 1×10^{-5} while 6 of them reached the standard genome-wide significance of 5×10^{-8}. We confirmed 11 SNPs that were reported in the original T2D GWAS analysis[23] and previous T2D related GWASs.[33,34] Another 7 SNPs that were reported to be associated with T2D-related traits were also confirmed in our analysis.[35,36] The remaining 15 SNPs were not previously reported in the original T2D GWAS[23] and the previous studies did not show their significance for T2D, while 2 SNPs of them showed high LD (r2 > 0.6) with the T2D-associated SNPs reported previously. For the 37 genes these 33 SNPs annotated to, 16 of them were newly detected compared to the original T2D[23] and previous T2D-related studies. The details are provided in Table S1. Of the detected loci for T2D, most of the genes were enriched in T2D-related terms “positive regulation of fatty acid oxidation”, and “white fat cell differentiation”. GO term enrichment analysis results are detailed in Table 2.

3.3. CARD gene loci identified with cFDR

Conditional on their association with T2D, we identified 34 significant SNPs (cFDR <0.05) for CARD variation (B in Fig. 2 and Table 3), which were located on 17 chromosomes (1–10,12–18) and annotated to 43 genes. In the original meta-analysis for CARD GWAS,[24] 18 SNPs had p-values smaller than 1×10^{-5} while 5 of them reached the standard genome-wide significance of 5×10^{-8}. We confirmed 13 SNPs that were reported in the original CARD GWAS analysis[24] and previous CARD related GWASs.[37,38] Another 5 SNPs that were reported to be associated with CARD-related traits were also confirmed in our analysis.[36,39] The other 16 SNPs were not previously reported in the original CARD GWAS[24] and the previous studies did not show their significance for CARD, and none of the novel SNPs showed high LD (r2 > 0.6) with the CARD-associated SNPs reported previously. For the 43 genes these 34 SNPs annotated to, there were 24 of them were newly detected compared to the original CARD[24] and previous CARD-related studies. The details are provided in Table S2. Of the detected loci for CARD, some of the genes were enriched in CARD-related terms “protein domain specific binding” and “lipoprotein lipase activity”. GO term enrichment analysis are detailed in Table 2.

3.4. Pleiotropic gene loci for both T2D and CARD

The conjunction FDR analysis detected 9 independent pleiotropic loci that were significantly (conjunction FDR < 0.05) associated with both traits (C in Fig. 2 and Table 4). Of the 9 identified pleiotropic variants, three SNPs rs10965212 *(CDKN2B-AS1),* rs4510208 *(ICA1L)* and rs10744777 *(ALDH2)* were reported to be significant for CARD in the original

CARD GWAS[24] or previous CARD GWAS.[38] The other two SNPs (rs11979110 and rs3843467) were previously reported to be associated with high density lipoprotein (HDL) and triglycerides.[36] The remaining four SNPs were not previously reported in the original T2D and CARD related GWASs and in the previous studies they were not significant for either T2D or CARD. For the 12 genes those pleiotropic SNPs annotated to, we found six of them *(CDKN2B-AS1, ICA1L, ALDH, RAI1, C5orf67* and *KLF14)* were reported by T2D or CARD related GWAS. The other six genes were not identified by any T2D or CAD related GWAS. For the SNPs that were annotated to these 6 genes, one SNP was located in the intronic regions of gene *CPPED1,* the rest of the SNPs were all located in intergenic regions of the genes. Detailed information were shown in Table 2. Of the detected 9 pleiotropic loci, most of the genes were enriched in T2D and CARD related terms “protein domain specific binding” and “negative regulation of multicellular organism growth”. Detailed information of GO term analysis is given in Table 2.

3.5. Protein-protein interaction network

The 37 identified T2D-associated genes were retrieved from the STRING database. Only 18 genes, including 3 novel genes, were annotated in this database. The 18 genes were clearly enriched in two clusters: *TCF7L2* and *HLA* (Fig. S3). Three novel genes *OASL, HLA-DQA2* and *HLA-DQB1,* respectively encoding 2′−5′-oligoadenylate synthetase like, major histocompatibility complex, class II, DQ alpha 2 and major histocompatibility complex, class II, DQ beta 1, were directly connected with the *HLA* cluster.

The 43 identified CARD-associated genes were retrieved from the STRING database. Only 4 genes, including 2 novel genes, were annotated in this database. The 4 genes were clearly enriched into two clusters: *ALDH2* and *LPL* (Fig. S4). Two novel genes, *ADH7* and *CDK8,* those respectively encoding alcohol dehydrogenase class 4 mu/sigma chains and cyclin-dependent kinase 8, were directly connected to the two clusters.

4. Discussion

In our study, two independent GWASs with summary statistic p values were combined to explore the pleiotropic enrichment of SNPs that are associated with T2D and CARD. Compared to the conventional standard single phenotype GWAS, simultaneously analyzing multiple related traits allows for the increased discovery of trait-associated variants without requiring additional larger datasets for individual trait. By leveraging the power of two different GWAS datasets from T2D and CARD, we discovered 33 loci for T2D and 34 loci for CARD. Using the standard GWAS significance in the datasets, only 6 for T2D and 5 for CARD were significant. Most of the genes have not been reported to show borderline significance with T2D and CARD respectively, as detailed in Tables S1 and S2. Adopting the genetic pleiotropic-informed cFDR method, we found 9 novel genes associated with both T2D and CARD. These novel findings may enable us to further dissect the overlapping genetic mechanisms between these two related phenotypes. The improved detection of novel susceptibility loci with genetic pleiotropy may lead us to a better understanding of common etiology between disorders and have a significant impact on the clinical treatment and prevention of related complex human diseases.

The cFDR approach was adopted here to account for some of the missing heritability between traits or diseases. This method employs the idea that a variant with significant effects in two associated phenotypes is more likely to be a true effect, and therefore has a higher probability of being detected in multiple independent studies. This technique allows for an increase in effective sample size and therefore a sub-sequent increase in power to detect true associations for more variants with small to moderate effect sizes which are often easily ignored in the standard single phenotype GWAS. In addition, the genetic enrichment presented in conditional Q-Q plots conveys that the decreased cFDR value for a given nominal p value greatly increases power to detect true association effects. When initially implementing the cFDR method, Andreassen et al.[7] demonstrated one advantage of this model-free empirical cdf approach is for the avoidance of bias in conditional FDR estimates from model misspecification, and they made a comparison of traditional unconditional FDR and cFDR methods, and found that the latter resulted in an increase of 15–20 times the number of SNPs under the same FDR threshold of 0.05.[7]

Our cFDR analysis identified 9 pleiotropic signals, which supported the close relationship and shared genetic determination between these two traits. These 9 pleiotropic SNPs were annotated to 12 genes. Five genes *CDKN2B-AS1, ICA1L, ALDH, C5orf67* and *RAH* were frequently reported and replicated in previous CARD related studies. The implementation of cFDR method in our study not only furnishes another empirical validation for the cFDR method to successfully detect novel and known disease associated genetic variants, but also shows the practicability of improved discovery of novel susceptibility loci using existing GWASs summary results. Six genes *(CDKN2B-AS1, ICA1L, ALDH, RAH, C5orf67* and *KLF14)* thatwere associated with either T2D or CARD in previous studies but not with both were detected as pleiotropic loci in this analysis. Furthermore, seven novel genes are worth noting because no previous study has reported associations with either T2D or CARD for them. For the SNPs that were annotated to these 6 genes, one SNP was located in the intronic regions of gene *CPPED1,* the rest SNPs were all located in intergenic regions of the genes. As examples, we will discuss gene *CPPED1* in the following for their potential functional relevance and significance.

The SNP rs4780476 is located at the intronic region of gene *CPPED1.* A study reported that the expression of *CPPED1* decreased after weight reduction in subcutaneous adipose tissue.[40] Moreover, *CPPED1* knockdown experiment demonstrated that *CPPED1* knockdown with small interfering RNA increased expression of genes involved in glucose metabolism and improved insulin-stimulated glucose uptake, which suggests the potential of *CPPED1* knockdown in the treatment of obesity-related phenotypes such as T2D.[40] We assume that this gene might be involved in certain processes that are significant in the development of T2D and CARD, however, more future studies are expected to explore the exact mechanisms of the novel gene we identified.

Our study presents several strengths. First, the statistical power is increased through the cFDR method by leveraging two large GWAS datasets, providing an increase in effective sample size. Although a meta-analysis of the same data would offer a similar gain, a meta-analysis only allows for more powerful detection of loci with the same direction of allelic effects in the phenotypes,[41] whereas the cFDR method allows for detecting loci regardless of

their effect directions. Secondly, we consider two traits that are unlikely to be correlated with T2D and CARD, ADHD and MDD, and generate conditional QQ plots with respect to these "control traits." This "control traits" enrichment analysis provides an alternative way to examine pleiotropic enrichment and provides a baseline that can be used to statistically partially validate the novel findings in our study. Our study may also have some limitations. First, we could not provide information about the effect estimates of pleiotropic loci on the phenotypes due to a lack of detailed individual-study-level data. However, we can infer this information from the summary beta values in the original GWAS study. This cFDR approach cannot distinguish between vertical and horizontal pleiotropy of the pleiotropic signals, although this question might be partially addressed in future summary-based Mendelian Randomization (SMR)[42,43] study. Second, it is likely that some of our cFDR results may be overstated due to overlapping samples although the model-free approach is able to neutralize this overestimation of the conservative cFDR estimate.[4,7,8] Alternative approaches may be applied to check whether novel loci could still be identified in order to further confirm novel findings in our study or to furnish an empirical comparison of the relative performance of alternative methods, a topic we wish to pursue in the future with comprehensive theoretical and simulation approaches.

In summary, by incorporating pleiotropic effects of two closely related traits into a conditional analysis framework, we observed significant pleiotropic enrichment between T2D and CARD, supporting the improved statistical power of the method. We identified several novel pleiotropic loci of potential functional significance for T2D and CARD in our analysis, and the results may provide us with novel insights into the shared genetic influences between these two disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research in context

T2D and CARD share primary risk factors such as smoking, hypertension, elevated lipid, dysbetalipoproteinemia and hyperglycemia, also some potential risk factors like obesity, lack of physical activity, cardiovascular family history, gender and age. We found additional common variants associated with T2D and CARD. We found 9 pleiotropic loci associated with both T2D and CARD. These findings may provide novel insights into the etiology of T2D and CARD, as well as the processes that may influence disease development both individually and jointly

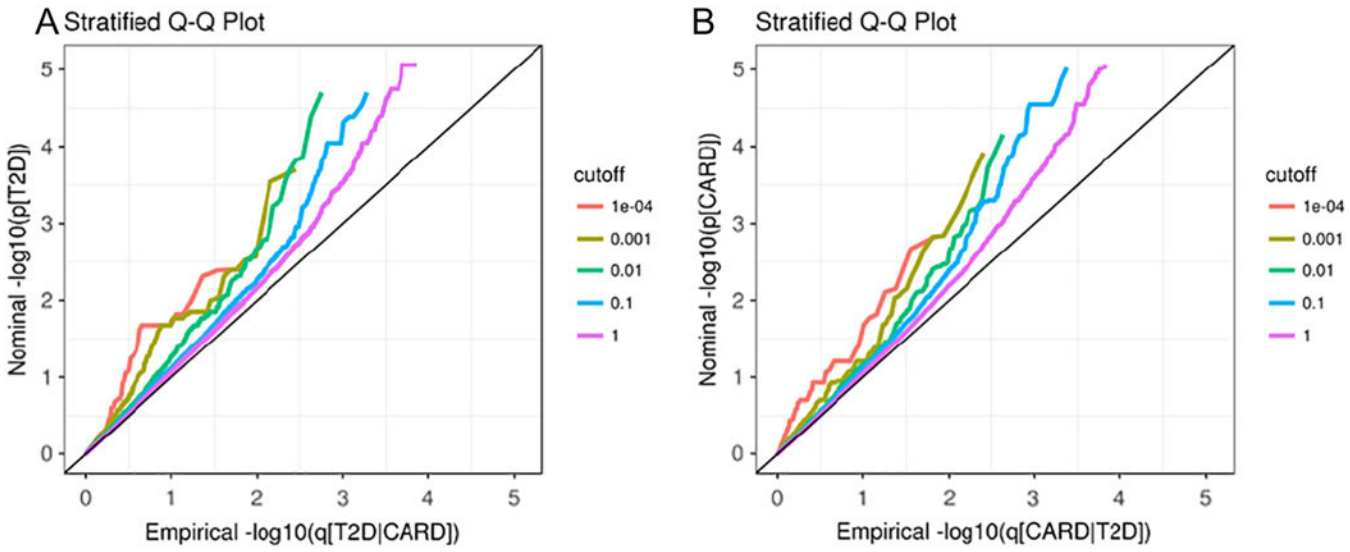

Fig. 1.

Stratified QQ plots. Stratified QQ plots of nominal *versus* empirical $-\log_{10}$ p-values in T2D (A) as a function of significance of the association with CARD, and in CARD (B) as a function of significance of the association with T2D. The purple line with slope of zero represents all SNPs.

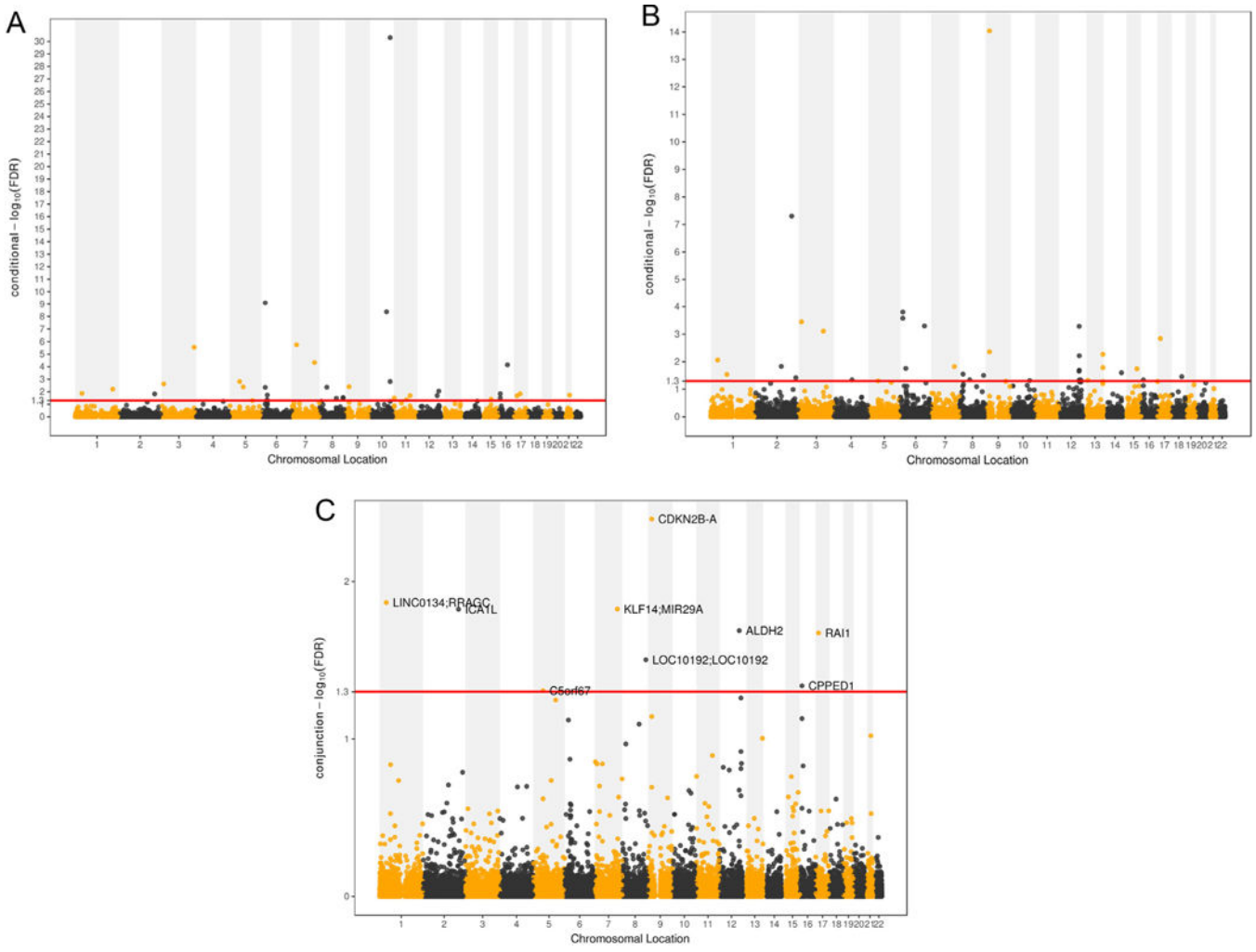

Fig. 2.

Conditional Manhattan plot of conditional $-\log_{10}$ FDR values for A) T2D given CARD (T2D|CARD), B) CARD given T2D (CARD|T2D), C) T2D and CARD. The red line marks the conditional $-\log_{10}$ FDR value of 1.3 corresponds to a cFDR <0.05.

Table 1

Conditional FDR value for T2D given the CARD (cFDR <.05).

RSID	ROLE	GENE	CHR	SNP type	Gene type	P.valueA	cFDR.AcB
rs10787472	Intronic	TCF7L2	chr10	Confirmed	Confirmed	1.10E-35	4.83E-31
rs6906327	Intronic	CDKAL1	chr6	Confirmed	Confirmed	3.10E-14	7.92E-10
rs7911264	Intergenic	KIF11,HHEX	chr10	Confirmed	Confirmed	4.50E-13	4.24E-09
rs849135	Intronic	JAZF1	chr7	Confirmed	Confirmed	3.40E-10	1.79E-06
rs4481184	Intronic	IGF2BP2	chr3	Confirmed	Confirmed	3.20E-10	2.83E-06
rs11979110	Intergenic	KLF14,MIR29A	chr7	HDL(24097068)	Confirmed, novel	1.00E-07	4.66E-05
rs9940128	Intronic	FTO	chr16	Confirmed	Confirmed	1.10E-08	7.15E-05
rs70797H	Intronic	TCF7L2	chr10	Novel	Confirmed	2.50E-07	0.001524
rs3843467	Intronic	C5orf67	chr5	HDL (24097068)	Novel	2.50E-06	0.001541
rs2881654	Intronic	PPARG	chr3	T2D(24509480)	Confirmed	1.70E-07	0.002381
rs10965212	ncRNA_intronic	CDKN2B-AS1	chr9	CARD (28530674)	Confirmed	0.004	0.004
rs6885904	ncRNA_intronic	ZBED3-AS1	chr5	Novel	Confirmed	1.10E-06	0.004178
rs516946	Intronic	ANK1	chr8	T2D (22885922)	Confirmed	7.30E-07	0.0044
rs4712540	Intronic	CDKAL1	chr6	Confirmed	Confirmed	5.80E-06	0.00453
rs340835	Intronic	PROX1	chr1	Fasting glucose (22885924)	Confirmed	1.10E-06	0.006194
rs7965349	Intronic	OASL	chr12	**LD (0.817 rs7957197 T2D)**	Novel	2.00E-05	0.00894
rs11211039	Intergenic	LINC01343,RRAGC	chr1	Novel	Novel, novel	2.00E-04	0.0136
rs4430796	Intronic	HNF1B	chr17	T2D (26551672)	Confirmed	2.40E-06	0.014504
rs4780476	Intronic	CPPED1	chr16	Novel	Novel	4.10E-05	0.014514
rs4510208	Intronic	ICA1L	chr2	CARD (26343387)	Novel	0.015	0.015
rs3892710	Intergenic	HLA-DQB1,HLA-DQA2	chr6	CARD (21971053)	Novel, novel	8.70E-06	0.018475
rs7280071	Intronic	RUNX1	chr21	Novel	Novel	4.40E-05	0.01881
rs10744777	Intronic	ALDH2	chr12	CARD (23202125)	CARD (23364394)	0.0041	0.0205
rs1876602	Intergenic	MTNR1B,SLC36A4	chr11	Novel	Confirmed, novel	4.10E-05	0.020739
rs3818717	Exonic	RAI1	chr17	Novel	CARD (24262325)	0.0049	0.021233
rs13275988	Intergenic	LOC101927798,LOC101927822	chr8	Novel	Novel, novel	0.00029	0.02987
rs1878016	Intronic	KCNQ3	chr8	Novel	CARD (23870195)	1.30E-05	0.030072
rs7l93741	Intronic	CPPED1	chr16	Novel	Novel	0.00014	0.031267

RSID	ROLE	GENE	CHR	SNP type	Gene type	P.valueA	cFDR.AcB
rs163177	Intronic	KCNQ1	chr11	T2D (26551672)	Confirmed	4.80E-05	0.0318
rs6991067	Intronic	INTS8	chr8	**LD (0.604 rs896854 T2D)**	Confirmed	0.00015	0.033188
rs1723839!	Intergenic	C2CD4B,MIR8067	chr15	Novel	Confirmed, novel	2.90E-05	0.039832
rs3l30931	UTR5	POU5F1	chr6	Novel	Confirmed	1.80E-05	0.043902
rs1783598	Intronic	FCHSD2	chr11	Novel	Novel	3.50E-05	0.043995

Notes:

SNP type means whether SNPs identified in our study compared to the original T22D GWAS and previous studies are Novel or Confirmed or associated with T2D-related traits (trait (PMID)) or in high LD with T2D-associated loci.

Gene type means whether genes identified in our study compared to the original T2D GWAS and previous studies are Novel or Confirmed.

P.valueA is the p value of T2D, A is T2D.

cFDRAcB is the cFDR value of T2D conditioned on CARD, B is CARD.

Table 2

Functional Term Enrichment Analysis.

Pathway ID	Pathway description	Count in gene set	False discovery rate
T2D GO:0002504	Antigen processing and presentation of peptide or polysaccharide antigen *via* MHC class II	14	5.533
GO:0061008	Hepaticobiliary system development	7	4.363
GO:0001889	Liver development	7	4.381
GO:0030855	Epithelial cell differentiation	10	3.249
GO:0070365	Hepatocyte differentiation	5	7.310
GO:0019904	Protein domain specific binding	18	3.425
GO:0016055	Wnt signaling pathway	10	3.842
GO:0048713	Regulation of oligodendrocyte differentiation	5	5.359
GO:0002674	Negative regulation of acute inflammatory response	4	6.895
GO:0046321	Positive regulation of fatty acid oxidation	4	6.648
GO:0050872	White fat cell differentiation	4	6.436
GO:0060214	Endocardium formation	3	7.895
GO:0070309	Lens fiber cell morphogenesis	3	7.895
GO:2000977	Regulation of forebrain neuron differentiation	3	8.158
GO:0042611	MHC protein complex	13	4.868
GO:0042613	MHC class II protein complex	13	6.530
GO:0098796	Membrane protein complex	17	2.256
GO:0098797	Plasma membrane protein complex	17	3.084
CARD GO:0019904	Protein domain specific binding	15	3.208
GO:0004465	Lipoprotein lipase activity	4	7.942
GO:0017129	Triglyceride binding	4	8.620
GO:0019433	Triglyceride catabolic process	4	6.418
T2D and CARD GO:0019904	Protein domain specific binding	14	5.173
GO:0040015	Negative regulation of multicellular organism growth	3	7.890

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Table 3

Conditional FDR value for CARD given the T2D (cFDR <.05).

RSID	ROLE	GENE	CHR	SNP type	Gene type	P.valueB	cFDR.BcA
rs10965212	ncRNA_intronic	CDKN2B-AS1	chr9	Confirmed	Confirmed	1.37E-17	9.10E-15
rs4510208	Intronic	ICA1L	chr2	Confirmed	Confirmed	4.29E-11	4.97E-08
rs9381462	Intronic	PHACTR1	chr6	Confirmed	Confirmed	5.13E-09	0.000157
rs2876303	Intronic	PHACTR1	chr6	Confirmed	Confirmed	9.10E-09	0.000264
rs7651039	Intronic	BTD	chr3	Confirmed	Confirmed	1.85E-08	0.000357
rs1029212	ncRNA_intronic	LINC01312,TARID	chr6	CARD (28530674)	Confirmed	6.23E-08	0.000504
rs10744777	Intronic	ALDH2	chr12	CARD (23202125)	Confirmed	1.52E-06	0.000519
rs2347252	Intronic	MRAS	chr3	CARD (23202125)	Confirmed	9.83E-08	0.000775
rs3818717	Exonic	RAI1	chr17	Novel	Confirmed	5.16E-06	0.001424
rs1011970	ncRNA_intronic	CDKN2B-AS1	chr9	Novel	Confirmed	6.37E-06	0.004352
rs4773144	Intronic	COL4A2	chr13	CARD (23202125)	Confirmed	4.15E-07	0.005338
rsll066301	Intronic	PTPN11	chr12	Total cholesterol (24097068)	Novel	5.20E-07	0.006047
rs11211039	Intergenic	LINC01343,RRAGC	chr1	Novel	Novel, novel	0.00012	0.008633
rs2252641	ncRNA_intronic	TEX41	chr2	CARD (23202125)	Confirmed	1.37E-05	0.014755
rs11979110	Intergenic	KLF14,MIR29A	chr7	HDL(20686565)	Novel, novel	0.002137	0.014959
rs9515203	Intronic	COL4A2	chr13	CARD (23202125)	Confirmed	3.42E-05	0.016238
rs2523414	ncRNA_exonic	LOC554223	chr6	Novel	Novel	2.83E-05	0.017416
rs4539564	Intergenic	ADAMTS7,MORF4L1	chr15	CARD (23202125)	Confirmed	9.46E-06	0.017974
rs17696736	Intronic	NAA25	chr12	Novel	Confirmed	4.12E-06	0.020291
rs7970490	Intronic	CUX2	chr12	Novel	Confirmed	2.18E-05	0.021993
rs2146238	Intronic	CYP46A1	chr14	Novel	Novel	2.61E-06	0.025067
rs894210	Intergenic	LPL,SLC18A1	chr8	Triglycerides (24097068)	Lipid (24386095), Triglycerides (24886709)	6.93E-05	0.028257
rs4415546	Intergenic	ZNF326,BARHL2	chr1	Novel	Novel, novel	5.56E-06	0.028883
rs13275988	Intergenic	LOC101927798,LOC101927822	chr8	Novel	Novel, novel	0.000647	0.031365
rs8089632	Intergenic	MALT1,ZNF532	chr18	Novel	Novel, novel	1.11E-05	0.034666
rs6713510	ncRNA_intronic	LOC646736	chr2	T2D (26551672)	Novel	9.77E-05	0.038066
rs6474069	ncRNA_intronic	LINC00968,LOC101929415	chr8	Novel	Novel, novel	9.83E-06	0.045441
rs4699748	Intergenic	ADH1C,ADH7	chr4	Novel	Novel, novel	7.84E-05	0.04562

RSID	ROLE	GENE	CHR	SNP type	Gene type	P.valueB	cFDR.BcA
rs2708081	Intronic	OASL	chr12	CARD (28530674)	Confirmed	0.000189	0.045881
rs4780476	Intronic	CPPED1	chr16	Novel	Novel	0.001434	0.045891
rs10774625	Intronic	ATXN2	chr12	Novel	Blood pressure (19430483)	7.19E-06	0.04631
rs9581678	Intergenic	CDK8,WASF3	chr13	Novel	Novel, novel	1.47E-05	0.047478
rs7902587	Intergenic	OBFC1,SLK	chr10	Novel	Novel, novel	7.07E-05	0.048121
rs3843467	Intronic	C5orf67	chr5	Triglycerides (24097068)	Novel	0.00705	0.049348

Notes:

SNP type means whether SNPs identified in our study compared to the original CARD GWAS and previous studies are Novel or Confirmed or associated with CARD-related traits (trait (PMID)).

Gene type means whether genes identified in our study compared to the original CARD GWAS and previous studies are Novel or Confirmed.

P.valueB is the p value of CARD, B is CARD.

cFDRBcA is the cFDR value of CARD conditioned on T2D, A is T2D.

Table 4

Conjunction FDR: Pleiotropic Loci in T2D and CARD (cFDR<0.05).

RSID	ROLE	GENE	CHR	P.valueA	P.valueB	cFDR.AcB	cFDR.BcA	conjunction FDR
rs10965212	ncRNA_intronic	CDKN2B-AS1	chr9	0.004	1.37E-17	0.004	9.10E-15	0.004
rs11211039	Intergenic	LINC01343,RRAGC	chr1	2.00E-04	0.00012	0.0136	0.0086328	0.0136
rs11979110	Intergenic	KLF14,MIR29A	chr7	1.00E-07	0.002137	4.66E-05	0.014959	0.014959
rs4510208	Intronic	ICA1L	chr2	0.015	4.29E-11	0.015	4.97E-08	0.015
rs10744777	Intronic	ALDH2	chr12	0.0041	1.52E-06	0.0205	0.00051908	0.0205
rs3818717	Exonic	RAI1	chr17	0.0049	5.16E-06	0.021233	0.00142416	0.02123333
rs13275988	Intergenic	LOC101927798,LOC101927822	chr8	0.00029	0.000647	0.02987	0.03136495	0.03136495
rs4780476	Intronic	CPPED1	chr16	4.10E-05	0.001434	0.014514	0.0458912	0.0458912
rs3843467	Intronic	C5orf67	chr5	2.50E-06	0.00705	0.001541	0.0493479	0.0493479

Notes:

P.valueA is the p value of T2D.

P.valueB is the p value of CARD.

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