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Increased identification of novel variants in type 2 diabetes, birth weight and their pleiotropic loci

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Abstract

Background—Clinical and epidemiological findings point to an association between type 2 diabetes (T2D) and low birth weight. However, the nature of the relationship is largely unknown. The aim of this study was to identify novel single nucleotide polymorphisms (SNPs) in T2D and birth weight, and their pleiotropic loci.

Methods—A pleiotropy-informed conditional false discovery rate (cFDR) method was applied to two independent genome-wide association studies (GWAS) summary statistics of T2D (n = 149 821) and birth weight (n = 26 836).

Results—A conditional Q–Q plot showed strong enrichment of genetic variants in T2D conditioned on different levels of association with birth weight. 133 T2D-associated SNPs, including 120 novel SNPs, were identified with a significance threshold of cFDR < 0.05; 13 significant birth weight-associated SNPs, including 12 novel SNPs (cFDR < 0.05) were identified. Conjunctional cFDR (ccFDR) analysis identified nine pleiotropic loci, including seven novel loci, shared by both T2D and birth weight (ccFDR < 0.05). Two novel SNPs located at the CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*; rs1012635; cFDR < 0.05) and adenylate cyclase 5 (*ADCY5*; rs4677887; cFDR < 0.05) genes are of note. These two genes increase the risk of T2D and low birth weight through the pathway of the "fetal insulin hypothesis."

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Disclosure

The authors declare that they have no conflicts of interest.

Conclusion—Several pleiotropic loci were identified between T2D and birth weight by leveraging GWAS results. The results make it possible to explain a greater proportion of trait heritability and improve our understanding of the shared pathophysiology between T2D and birth weight.

Keywords

birth weight; genome-wide association study; pleiotropy; type 2 diabetes

Introduction

Diabetes is a group of systemic metabolic disorders characterized by long-term hyperglycemia due to scarcity of insulin secretion and/or deficiency of insulin action.[1] Diabetes is an increasingly serious global public health issue. Type 2 diabetes (T2D), the most common form of diabetes, begins with a condition of insulin resistance in which target cells fail to respond to insulin properly.[2] Currently, more than 400 million people throughout the world suffer from diabetes.[3] Over 90% of cases are T2D,[4] which caused 4.9 million deaths in 2014.[5] Type 2 diabetes has become one of leading causes of death, disability, and increased health care costs.

There is accumulating evidence showing that early life experiences, such as birth weight, have continuous effects on adult health.[6] Numerous studies have demonstrated that low birth weight can lead to increased risk of developing T2D. For example, a prospective study including three large cohorts (n = 149 794) found that participants with low birth weight (2.00–2.75 kg) had significantly higher susceptibility to T2D than those with reference birth weight (3.25–3.75 kg).[7] A recent meta-analysis of two studies analyzed 3627 T2D cases and 12 974 control participants of European ancestry.[8] A genetic risk score was created on the basis of five low birth weight-related single nucleotide polymorphisms (SNPs). The analysis showed that for each 1 point increment in the genetic risk score, the risk of developing T2D increased by 6%.[8] Using Mendelian randomization, it was further demonstrated that the low birth weight was actually causing the excess risk in T2D.[8]

Previous genome-wide association studies (GWAS) have been successful in identifying potential genetic risk factors for T2D that are associated with birth weight.[9] Despite a number of SNPs found to be reproducibly associated with both traits, these SNPs explain a small proportion of heritability for T2D (<5%) and birth weight (~25%),[10,11] and new information on the nature of the genetic component of these phenotypes needs to be investigated. In order to explain a greater proportion of genetic mechanisms underlying these highly correlated phenotypes in the pathogenesis of T2D and birth weight, further innovative analytical methods are required to uncover additional novel genes or variants, especially novel shared variants associated with both T2D and birth weight. As a novel analytical approach, the pleiotropy-informed conditional false discovery rate (cFDR) method,[12] which requires only summary statistics from GWAS, could provide increased power to detect SNPs in GWAS results and elucidate mechanistic relationships between genetically related phenotypes. Using this approach, Andreassen et al.[12] reported genetic overlap between a

number of diseases and phenotypes, and successfully identified common variants associated with schizophrenia and bipolar disorder.

In the present study we applied the cFDR method to two large and independent T2D and birth weight GWAS datasets[13,14] to identify additional and novel genetic loci of these two traits and to determine whether T2D shares susceptibility loci with birth weight, with the aim of gaining insights into the common pathophysiology between T2D and birth weight.

Methods

Genome-wide association studies datasets

The GWAS summary statistics were obtained from two publicly available datasets. The Diabetes Genetics Replication and Meta-analysis (DIAGRAM) consortium dataset (http://diagram-consortium.org/downloads.html, accessed 1 January 2015) contains the results for association with T2D from 34 840 cases and 114 981 controls. To our knowledge, it is the largest meta-analysis data of the reported T2D GWAS studies at present. The Early Growth Genetics Consortium dataset (http://egg-consortium.org/birth-weight.html, accessed 1 January 2015) contains the results for association with birth weight from a meta-analysis of up to 18 population-based European studies including 26 836 subjects. The two datasets contain summary statistics, providing *P*-values, size, and direction of effects at over 2 million directly genotyped or imputed SNPs from the HapMap project (release 27; ftp://ftp.ncbi.nlm.nih.gov/hapmap/00README.releasenotes_rel27, accessed 10 February 2015). There were no overlapping subjects between the T2D and birth weight GWAS datasets. The detailed inclusion criteria and phenotype characteristics from different GWAS are described in the original publications.[13,14]

Data processing

Two combined GWAS meta-analysis summary statistics for the 95 861 common SNPs were annotated, and then pairs of SNPs with large correlations were removed using the linkage disequilibrium (LD)-based pruning method. The LD pruning method begins with a window of 50 SNPs where the LD between each pair of SNPs is calculated, and if pairs have an $R^2 > 0.2$, one of that pair of SNPs (the SNP with the smaller minor allele frequency) is removed. Following this initial removal of SNPs, the window shifts five SNPs forward and the procedure is repeated until there is no pair of SNPs in high LD. The HapMap 3 genotypes (http://www.sanger.ac.uk/resources/downloads/human/hapmap3.html, accessed 15 August 2015) were used to prune the dataset. At the end of the pruning procedure, there were 32 132 variants remaining to be used in the analysis. Genomic control is often needed to adjust GWAS results to ensure that the variance estimates for each SNP are not inflated due to population structure. Genomic control was previously applied by the original authors in the two datasets,[13,14] so there was no need to reapply this adjustment in the present study.

Statistical analysis

Pleiotropic enrichment estimation—In GWAS studies, quantile–quantile (Q–Q) plots are commonly used to show the observed association (*y*-axis) across SNPs compared with the expected distribution of association test statistics (*x*-axis) under the null hypothesis. Any

deviation from the identity line implies either incorrect assumed distribution or a true association. In the present study, to estimate the pleiotropic enrichment of association compared with that expected under the null hypothesis, conditional Q–Q plots were constructed by successively conditioning the principal trait on the SNPs with varying strengths of association in the conditional trait as per Andreassen et al.[12] The Q–Q curves were plotted for quantiles of nominal $-\log_{10}(P)$ values for association of the subset of variants below each significance threshold in the conditional trait. Specifically, nominal *P*-values ($-\log_{10}(P)$) were plotted on the *y*-axis and empirical quantiles ($-\log_{10}(q)$) were plotted on the *x*-axis for T2D and birth weight, respectively. Pleiotropy enrichment can be assessed from the degree of leftward shift from the expected identity line as the principal phenotype is successively conditioned on more stringent significance criteria in the conditional phenotype. Greater spacing between conditional Q–Q curves intuitively indicates a stronger trend of pleiotropic enrichment shared between the principal and conditional traits.

Calculation of the cFDR—In order to identify novel loci associated with T2D and birth weight, the cFDR was computed, an extension of the standard FDR framework that incorporates GWAS summary statistics from the pruned dataset to demonstrate the probability that a random SNP is null for association with the principal phenotype given that the observed *P*-values for the principal and conditional phenotypes are both smaller than two predefined disease-specific significance thresholds.[15] In the present study, the cFDR was calculated for each SNP where T2D was the principal phenotype conditioned on the strength of association with birth weight (T2D|birth weight) and vice versa (birth weight|T2D). To assess whether the cFDR method leads to enrichment of specific loci, we successively confined the subset of SNPs being tested based on the level of significance for the association of each variant with the conditional trait using the following criteria: $P < 1$ (all SNPs), $P < 0.1$, $P < 0.01$, and $P < 0.001$. A cFDR of 0.05 was used to distinguish whether an SNP is significantly associated with the principal phenotype. The procedures used are described in detail by Andreassen et al.[12] To visualize the localization of significant loci associated with T2D given their association with birth weight and vice versa, a cFDR Manhattan plot was constructed, which marks the significance of various SNPs and their chromosomal locations.

Calculation of conjunctional cFDR—To determine pleiotropic loci, the conjunctional cFDR (ccFDR) was calculated, which refers to the possibility that a given SNP has a false positive association with both the principal and conditional traits. The ccFDR was computed as the maximum cFDR values (i.e. T2D| birth weight and birth weight|T2D) of the two traits. A ccFDR of 0.05 was used to identify whether an SNP is a pleiotropic locus. To visualize the localization of significant pleiotropic loci, ccFDR Manhattan plots were constructed on the basis of the ranking of ccFDR.

Function annotation of pleiotropic SNPs

To explore whether any of the identified pleiotropic SNPs may play a functional role in T2D and birth weight, each pleiotropic SNP was annotated to corresponding DNA features or regulatory elements in non-coding regions using HaploReg (http://www.broadinstitute.org/

mammals/haploreg/haploreg.php, acc essed 1 March 2016) and RegulomeDB (http://www.regulomedb.org/, accessed 15 March 2016) tools. HaploReg retrieves the ENCODE annotation for the SNP of interest as well as other SNPs in LD; the user can configure values (such as the LD threshold and the reference population used) from 1000 genomes datasets. RegulomeDB retrieves the ENCODE annotation and calculates a score for the regulatory potential of this region. It also has a database of predicted functional SNPs, by disease or trait and by SNP. Then, the program GOEAST (http://omicslab.genetics.ac.cn/GOEAST/, accessed 10 May 2016) was used to identify significantly enriched gene ontology (GO) terms among the list of genes associated with pleiotropic SNPs. The *P*-values were calculated by hypergeometric tests and adjusted for multiple comparisons by stringent Yekutieli (FDR under dependency) adjustment.[16] In order to partially explore and characterize the functional relationship of the T2D genes identified, the corresponding protein association networks were constructed using the STRING 10.0 database (http://string-db.org/, accessed 1 May 2016).

Results

Pleiotropic enrichment of T2D SNPs conditional on association with birth weight and vice versa

Conditional Q–Q plots are a common method to graphically assess the pleiotropic enrichment of genetic loci. Interestingly, we observed a strong enrichment of T2D-associated SNPs, with the proportion of true effects in T2D varying considerably depending on different levels of association for birth weight (Fig. 1a) because there appears to be a greater amount of separation between the different curves. As shown in Fig. 1b, there is a less robust enrichment pattern for birth weight conditioned on T2D compared with the pattern for T2D conditioned on birth weight. The presence of an earlier leftward shift indicates a greater proportion of true associations for the principal trait given the nominal *P*-value of the conditional trait.

Type 2 diabetes loci identified with cFDR

As shown in the cFDR Manhattan plot for T2D conditioned on birth weight (see Fig. S1, available as Supplementary Material to this paper), 133 significant SNPs were identified with a significance threshold of cFDR < 0.05 on 21 different chromosomes (Table S1). Interestingly, 13 of these significant SNPs reached genome-wide significance at 5×10^{-8} in the original meta-analysis for T2D,[13] including two loci (rs2881654 and rs2283228) also reported in previous T2D GWAS.[17,18] More importantly, 120 novel loci were identified that had been overlooked in the original meta-analysis.[13] Using the more conservative threshold of cFDR < 0.01, 62 significant loci remained (Table S1). Interestingly, there were five significant SNPs, including three novel SNPs rs163177 (cFDR = 1.50×10^{-8}), rs234857 (cFDR = 3.47×10^{-3}) and rs3852527 (cFDR = 4.73×10^{-3}) located at the potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) gene (11p15.4), that were associated with an increased risk of T2D susceptibility in previous studies.[18,19] To explore the functional association among identified T2D target genes and networks involved in the biological function of T2D, the genes that include the 133 significant SNPs were uploaded into the STRING 10.0 database. Interestingly, the network consisted of positive regulation of

cellular process genes and negative regulation of transcription from RNA polymerase II promoter genes, showing a strong protein– protein interaction among proteins corresponding to the T2D target genes (Fig. 2).

Birth weight loci identified with cFDR

As shown in the cFDR Manhattan plot for birth weight (Fig. S2), 13 significant SNPs were identified with a significance threshold of cFDR < 0.05 for birth weight variation on their association with T2D; these were mapped to nine different chromosomes (Table 1). Using the more conservative threshold of conditional cFDR < 0.01, three significant loci remained. Of interest, the current pleiotropy-informed cFDR method validated a locus (rs1042725) that was identified in a previous birth weight GWAS study.[14] Twelve new loci were discovered for the 13 significant SNPs at cFDR <0.05, including a novel SNP rs1012635 observed at the previously identified T2D-associated gene CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*).[20]

Pleiotropic loci in T2D and birth weight identified with ccFDR

To investigate whether any of the SNPs associated with T2D conditioned on birth weight were also significantly associated with birth weight conditioned on T2D, ccFDR was calculated and a ccFDR Manhattan plot was constructed (Fig. 3). Nine independent pleiotropic SNPs on a total of seven chromosomes reached a significance level of ccFDR < 0.05 (Table 2). Of the nine independent pleiotropic SNPs, rs231354 has previously been associated with T2D[21] and rs1042725 has been associated with both T2D and birth weight.[14,22] The present analysis reports seven novel pleiotropic SNPs not previously detected. These seven loci annotated at eight different genes, of which six (cytoplasmic polyadenylation element binding protein 3 [*CPEB3*], PBX homeobox 4 [*PBX4*], adrenoceptor beta 1 (*ADRB1*), melatonin receptor 1B [*MTNR1B*], solute carrier family 36 member 4 [*SLC36A4*], and bromodomain containing 1 [*BRD1*]) are novel genes and another two genes (*CDKAL1* and adenylate cyclase 5 [*ADCY5*]) were reported in previous T2D and birth weight GWAS.[9]

Functional and pathway analysis for pleiotropic loci

A series of bioinformatics analyses was conducted to explore the potential regulatory functions for nine pleiotropic SNPs. As annotated using HaploReg and RegulomeDB databases, rs1012635 (*CDKAL1*), rs1042725 (high mobility group AT-hook 2 [*HMGA2*]), rs12610185 (*PBX4*), rs231354 (*KCNQ1*), rs4753073 (*MTNR1B*) and rs2782980 (*ADRB1*) overlapped with open chromatin in a number of ENCODE cell lines, such as hepatocyte, fibroblast and osteoblast cell lines, which potentially related to T2D or birth weight phenotypes. Furthermore, we identified six pleiotropic SNPs, namely rs12610185 (*PBX4*), rs231354 (*KCNQ1OT1*), rs2782980 (*ADRB1*), rs4677887 (*ADCY5*), rs4753073 (*MTNR1B*) and rs916419 (*BRD1*), that fell into the enhancer regions of the corresponding genes annotated to these SNPs in a number of organs, such as the liver, pancreas, colonic mucosa and small intestine, confirming that these pleiotropic SNPs are highly enriched within regions of active chromatin state. To systematically investigate whether the observed pleiotropic SNPs were T2D and birth weight specific, GO analysis was conducted (Table 3), revealing a variety of biological processes, (e.g. adenosine receptor signaling pathway [P =

4.29×10^{-6}], G-protein-coupled purinergic receptor signaling pathway [$P = 4.29 \times 10^{-6}$] and adenylate cyclase-activating dopamine receptor signaling pathway [$P = 9.79 \times 10^{-6}$]) that are closely related to T2D and birth weight.

Discussion

By applying the stratified cFDR method to the summary statistics of T2D and birth weight GWAS, 133 T2D susceptibility loci were identified, including 120 novel loci that were missed in the original GWAS meta-analysis for T2D.[13] Furthermore, 13 significant birth weight-associated SNPs conditioned on T2D (cFDR < 0.05) were identified that were mapped to nine different chromosomes. Importantly, nine pleiotropic SNPs were identified suggesting a shared genetic mechanism among them. The results demonstrate that GWAS from birth weight may improve discovery of T2D susceptibility loci and enhance our understanding of the effect of common genetic variants on both traits.

In the present study, the most significant novel T2D susceptibility SNP identified by the cFDR method was rs163177. This SNP is located at the T2D risk gene *KCNQ1* (11p15.4). The *KCNQ1* gene, encoding the voltage-gated K^+ channel KvLQT1 subunit,[23] regulates insulin secretion function through the voltage-gated K^+ channel, which drives an electrical signal in pancreatic β-cells to promote glucose-stimulated insulin release.[24] In addition, Torekov et al. reported that *KCNQ1* long QT syndrome patients are susceptible to symptomatic hypoglycemia and hyperinsulinemia, because *KCNQ1* gene mutations can delay repolarization of β-cells and result in increased insulin secretion.[25] Another interesting SNP, rs4848526 (2q14.2), is located in the intron of the engrailed homeobox 1 (*EN1*) gene, which plays an important role in WNT signaling activation.[26] The WNT signaling pathway is associated with many physiological and pathophysiological activities, including T2D.[27] It is involved in the pathogenesis of T2D by regulating β-cell genesis and proliferation,[28] modulating lipid metabolism and insulin secretion,[29] and mediating the production of the incretin hormone glucagon-like peptide-1 (GLP-1).[30] Based on known and predicted protein–protein interactions, network analysis of the T2D target genes further illustrated the interactions of molecules that regulate metabolism of T2D and clustered these genes into functional categories. Interestingly, the network clusters that involved positive regulation of cellular process, negative regulation of transcription from RNA polymerase II promoter, positive regulation of lipid metabolic process, hepatoblast differentiation, and hepatocyte differentiation may be the functionally regulated modules for pathophysiology of T2D.

Furthermore, the current pleiotropy cFDR method identified 13 significant SNPs for birth weight conditioned on T2D (cFDR < 0.05), including SNP rs1042725, which was reported to be associated with birth weight in a previous GWAS.[14] Twelve new loci were discovered. For example, the novel significant SNP rs1012635 was annotated at gene *CDKAL1*, which showed a strong association with birth weight in a previous study.[20] The *CDKAL1* gene is located on chromosome 6p22.3, is 698 kbp long and encodes a member of the methylthiotransferase family. Previous studies suggested that *CDKAL1* was expressed in human pancreatic islets and may be associated with insulin secretion by pancreatic β-cells by interacting with cyclin dependent kinase 5 (*CDK5*).[31] However, the exact function of *CDKAL1* remains unclear.

Importantly, nine pleiotropic variants were identified and the SNP rs1042725 in *HMGA2* (12q14.3) has been associated with both T2D and birth weight.[14,22] As an important genetic determinant of birth weight and human height,[14,32] *HMGA2* was also considered a T2D risk gene.[33] In white adipose tissue, high expression of *HMGA2* can drive cellular senescence to increase susceptibility to T2D.[33] More importantly, seven novel pleiotropic loci were identified (rs1012635 in *CDKAL1*, rs10882028 in *CPEB3*, rs12610185 in *PBX4*, rs2782980 in *ADRB1*, rs4677887 in *ADCY5*, rs4753073 in *MTNR1B* [*SLC36A4*] and rs916419 in *BRD1*) that have not been reported in previous studies. Both *CDKAL1* and *ADCY5* were confirmed to increase risk of T2D and low birth weight through the "fetal insulin hypothesis,"[9] suggesting that gene variation related to pancreatic β-cell function or insulin secretion during embryonic development can lead to decreased birth weight as well as subsequent development of T2D.[9] The GO analysis revealed an enrichment of biological processes that are closely related to T2D and birth weight, including the adenosine receptor signaling pathway and the G-protein-coupled purinergic receptor signaling pathway, among others. A previous study reported that the adenosine A_{2B} receptor signaling pathway modulates glucose, lipid homeostasis and chronic inflammation, and regulates the activity of resident macrophages in adipose tissue in T2D.[34] Interestingly, changes in cardiac glucose metabolism are associated with low birth weight,[35] suggesting a potential role for the adenosine receptor in low birth weight.

The major strengths of the present study are that by leveraging of GWAS results from T2D and birth weight phenotypes, we successfully improved the detection of uncovered T2D associated loci without additional large datasets and identified several novel pleiotropic SNPs using the cFDR method. These findings present novel insights for exploring common underlying molecular mechanisms and offer promising clues for further experimental studies. There may be some limitations to the present study. First, we were unable to associate the genetic findings with clinical outcomes due to our inability to access raw clinical data. However, the primary purpose of the study was to improve the identification of disease-associated genes and explore the overlapping biological mechanisms between T2D and birth weight. In addition, we did not identify all the previously implicated genes in T2D and birth weight because the present study analyzed only a subset of available GWAS to efficiently reveal the missing heritability in the two traits. Therefore, clinical replications and further biological experiments are necessary to validate our findings.

In conclusion, the present study demonstrated high efficiency of the cFDR method in improving the identification of novel genetic variants of both T2D and birth weight. The findings offer novel insights into potential shared genetic mechanisms in T2D and birth weight, which may form a basis for further biological experiments and clinical replication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- This study demonstrates high efficiency of the conditional false discovery rate (cFDR) method in improving the identification of novel genetic variants of both type 2 diabetes (T2D) and birth weight.
- The findings offer novel insights into potential shared genetic mechanisms in T2D and birth weight, which may form a basis for further biological experiments and clinical replication.
- The study identified two novel pleiotropic loci that may be related to the processes that affect T2D metabolism and may therefore contribute to the genetic susceptibility to T2D.

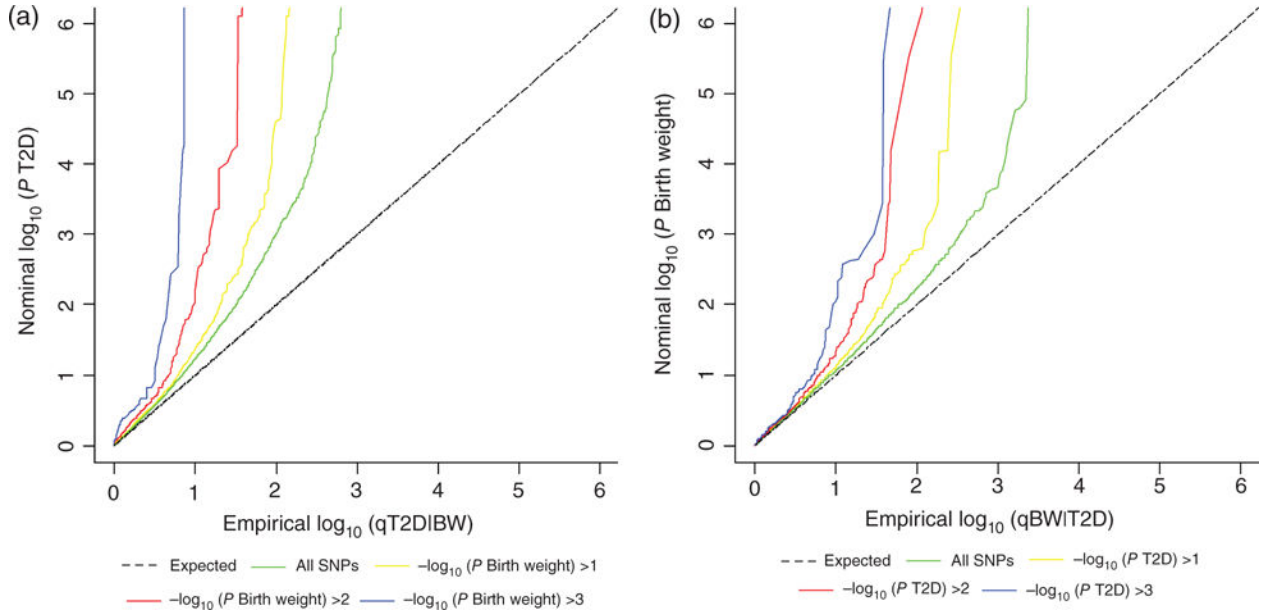

Figure 1.

Conditional Q–Q plot. Stratified Q-Q plot of enrichment versus nominal $-\log_{10}$ *P*-values (corrected for inflation) in (a) type 2 diabetes (T2D) as a function of significance of the association with birth weight (T2D|birth weight) and (b) birth weight as a function of significance of the association with T2D (birth weight|T2D) below the standard genome-wide association study threshold of $P < 5 \times 10^{-8}$ at the level of $-\log_{10}(P) > 0$, $-\log_{10}(P) > 1$, $-\log_{10}(P) > 2$, and $-\log_{10}(P) > 3$ corresponding to $P < 1$, $P < 0.1$, $P < 0.01$, and $P < 0.001$, respectively. Dashed lines indicate the null-hypothesis.

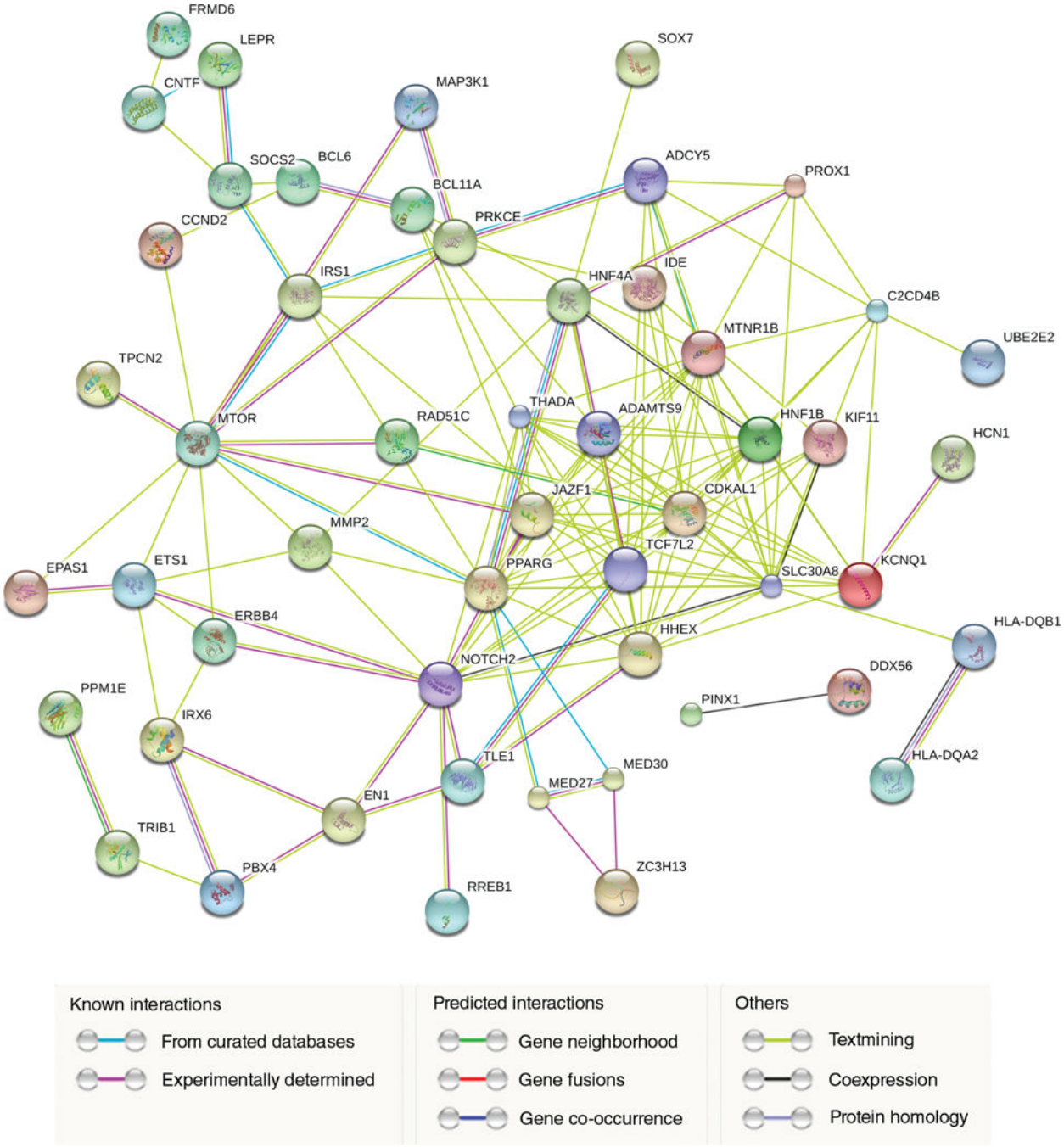

Figure 2.

Functional protein association network analysis for type 2 diabetes (T2D) susceptibility genes. Connections are based on coexpression and experimental evidence with a STRING 10.0 (http://string-db.org/, accessed 1 May 2016) summary score above 0.4. The network that related to positive regulation of cellular process and negative regulation of transcription from RNA polymerase II promoter showed significant enrichment for T2D susceptibility genes. Each filled node denotes a gene; edges between nodes indicate protein–protein interactions between protein products of the corresponding genes. Different edge colors

represent the types of evidence for the association. Definitions for all protein symbols are given in Table S2.

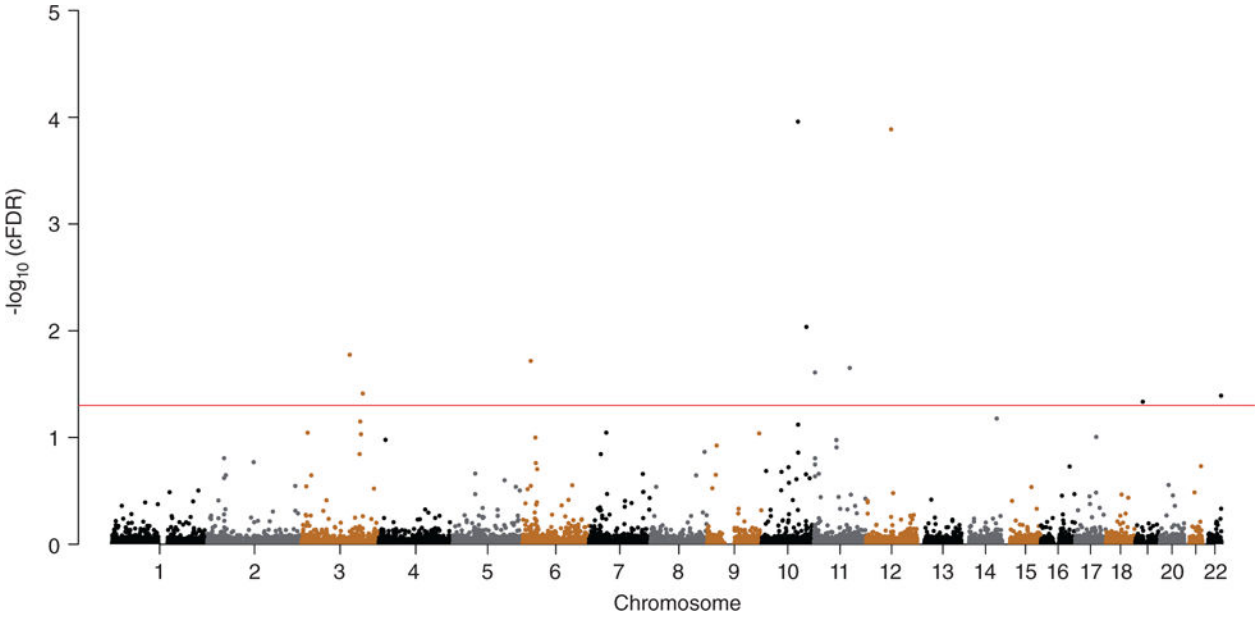

Figure 3.

"Conjunctional Manhattan plot" of conjunctional $-\log_{10}$ (conditional false discovery rate [cFDR]) values for type 2 diabetes (T2D) and birth weight. Single nucleotide polymorphisms with conjunctional $-\log_{10}$ cFDR >1.3 (i.e. cFDR < 0.05) are shown above the red line. The figure marks the chromosomal locations of significant loci. Details for all significant loci are given in Table 2.

Table 1

Conditional false discovery rate: birth weight loci given type 2 diabetes (birth weight|T2D)

SNP	SNP chromosome	SNP position	Birth weight *P*-value	cFDR (birth weight|T2D)	Nearby gene
rs1012635	6	20783274	4.10×10^{-3}	1.91×10^{-2}	CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*)
rs1042725	12	64644614	2.80×10^{-9}	2.21×10^{-7}	High mobility group AT-hook 2 (*HMGA2*)
rs10882028	10	93933880	2.90×10^{-6}	1.07×10^{-4}	Cytoplasmic polyadenylation element binding protein 3 (*CPEB3*)
rs12610185	19	19582722	7.70×10^{-3}	4.62×10^{-2}	PBX homeobox 4 (*PBX4*)
rs231354	11	2662927	2.30×10^{-3}	2.45×10^{-2}	Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)
rs2782980	10	115771517	6.40×10^{-5}	8.30×10^{-3}	Adrenoceptor beta 1 (*ADRB1*)
rs4677887	3	124582913	3.60×10^{-4}	1.67×10^{-2}	Adenylate cyclase 5 (*ADCY5*)
rs4753073	11	92357123	2.70×10^{-3}	2.23×10^{-2}	Melatonin receptor 1B (*MTNR1B*)||solute carrier family 36 member 4 (*SLC36A4*)
rs916419	22	48555425	3.50×10^{-4}	4.05×10^{-2}	Bromodomain containing 1 (*BRD1*)
rs871961	3	150061208	6.70×10^{-5}	4.26×10^{-2}	Carboxypeptidase B1 (*CPB1*)||carboxypeptidase A3 (*CPA3*)
rs1258191	10	50663125	1.20×10^{-5}	2.71×10^{-2}	Mitogen-activated protein kinase 6 pseudogene 6 (*MAPK6PS6*)|| *LOC727726*
rs6016373	20	38587509	1.60×10^{-5}	4.06×10^{-2}	Heat shock protein family E (Hsp10) member 1 pseudogene 1 (*HSPE1P1*)||MAF bZIP transcription factor B (*MAFB*)
rs222857	17	7105287	1.70×10^{-5}	4.02×10^{-2}	Claudin 7 (*CLDN7*)

*The chromosome position in the GRCh37.p5 sequence of Genome Build 37.3 (ftp://ftp.ncbi.nlm.nih.gov/genomes/Homo_sapiens/ARCHIVE/BUILD.37.3, accessed 1 May 2015).

SNP, single nucleotide polymorphism; cFDR, conditional false discovery rate.

Table 2

Conjunctional conditional false discovery rate: pleiotropic loci in type 2 diabetes and birth weight

			cFDR			
SNP	**SNP chromosome**	**SNP position***	**T2D|birth weight**	**Birth weight|T2D**	**ccFDR**	**Nearby gene**
rs1012635	6	20783274	3.36×10^{-8}	1.91×10^{-2}	1.91×10^{-2}	CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*)
rs1042725	12	64644614	1.29×10^{-4}	2.21×10^{-7}	1.29×10^{-4}	High mobility group AT-hook 2 (*HMGA2*)
rs10882028	10	93933880	1.10×10^{-4}	1.07×10^{-4}	1.10×10^{-4}	Cytoplasmic polyadenylation element binding protein 3 (*CPEB3*)
rs12610185	19	19582722	2.35×10^{-5}	4.62×10^{-2}	4.62×10^{-2}	PBX homeobox 4 (*PBX4*)
rs231354	11	2662927	2.94×10^{-5}	2.45×10^{-2}	2.45×10^{-2}	Potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*)
rs2782980	10	115771517	9.18×10^{-3}	8.30×10^{-3}	9.18×10^{-3}	Adrenoceptor beta 1 (*ADRB1*)
rs4677887	3	124582913	3.68×10^{-3}	1.67×10^{-2}	1.67×10^{-2}	Adenylate cyclase 5 (*ADCY5*)
rs4753073	11	92357123	2.67×10^{-5}	2.23×10^{-2}	2.23×10^{-2}	Melatonin receptor 1B (*MTNR1B*)||solute carrier family 36 member 4 (*SLC36A4*)
rs916419	22	48555425	2.11×10^{-2}	4.05×10^{-2}	4.05×10^{-2}	Bromodomain containing 1 (*BRD1*)

*The chromosome position in the GRCh37.p5 sequence of Genome Build 37.3 (ftp://ftp.ncbi.nlm.nih.gov/genomes/Homo_sapiens/ARCHIVE/BUILD.37.3, accessed 1 May 2015).

SNP, single nucleotide polymorphism; cFDR, conditional false discovery rate; ccFDR, conjunctional cFDR; T2D, type 2 diabetes.

Table 3

Top five most significant gene ontology terms enriched for genes associated with pleiotropic SNPs

GO ID	GO terms	log (OR)	*P*-value
GO:0001973	Adenosine receptor signaling pathway	9.66	4.29×10^{-6}
GO:0035588	G-Protein-coupled purinergic receptor signaling pathway	9.66	4.29×10^{-6}
GO:0007195	Adenylate cyclase-inhibiting dopamine receptor signaling pathway	9.14	9.79×10^{-6}
GO:0035587	Purinergic receptor signaling pathway	8.66	2.43×10^{-5}
GO:0007191	Adenylate cyclase-activating dopamine receptor signaling pathway	8.47	3.42×10^{-5}

Gene ontology (GO) enrichment analysis was performed using the GOEAST program (http://omicslab.genetics.ac.cn/GOEAST/, accessed 10 May 2016), which gave "$P > 0$" when the obtained *P*-value was less than the minimum float value ($1.17549435082229 \times 10^{-38}$). OR, odds ratio.