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Review

Shared genetic etiology underlying Alzheimer's disease and type 2 diabetes

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ABSTRACT

Epidemiological evidence supports the observation that subjects with type 2 diabetes (T2D) are at higher risk to develop Alzheimer's disease (AD). However, whether and how these two conditions are causally linked is unknown. Possible mechanisms include shared genetic risk factors, which were investigated in this study based on recent genome wide association study (GWAS) findings. In order to achieve our goal, we retrieved single nucleotide polymorphisms (SNPs) associated with T2D and AD from large-scale GWAS meta-analysis consortia and tested for overlap among the T2D- and AD-associated SNPs at various p-value thresholds. We then explored the function of the shared T2D/AD GWAS SNPs by leveraging expressional quantitative trait loci, pathways, gene ontology data, and co-expression networks. We found 927 SNPs associated with both AD and T2D with p-value ≤0.01, an overlap significantly larger than random chance (overlapping p-value of 6.93E–28). Among these, 395 of the shared GWAS SNPs have the same risk allele for AD and T2D, suggesting common pathogenic mechanisms underlying the development of both AD and T2D. Genes influenced by shared T2D/AD SNPs with the same risk allele were first identified using a SNP annotation variation (ANNOVAR) software, followed by using Association Protein-Protein Link Evaluator (DAPPLE) software to identify additional proteins that are known to physically interact with the ANNOVAR gene annotations. We found that gene annotations from ANNOVAR and DAPPLE significantly enriched specific KEGG pathways pertaining to immune responses, cell signaling and neuronal plasticity, cellular processes in which abnormalities are known to contribute to both T2D and AD pathogenesis. Thus, our observation suggests that among T2D subjects with common genetic predispositions (*e.g.*, SNPs with consistent risk alleles for T2D and AD), dysregulation of these pathogenic pathways could contribute to the elevated risks for AD in subjects. Interestingly, we found that 532 of the shared T2D/AD GWAS SNPs had divergent risk alleles in the two diseases. For individual shared T2D/AD SNPs with divergent alleles, one of the allelic forms may contribute to one of the diseases (*e.g.*, T2D), but not necessarily to the

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other (*e.g.*, AD), or vice versa. Collectively, our GWAS studies tentatively support the epidemiological observation of disease concordance between T2D and AD. Moreover, the studies provide the much needed information for the design of future novel therapeutic approaches, for a subpopulation of T2D subjects with genetic disposition to AD, that could benefit T2D and reduce the risk for subsequent development of AD.

Contents

1. Introduction

An estimated 347 million people worldwide suffer from diabetes, with 90% of this population (312 million) suffering specifically from type 2 diabetes (T2D) (World Health Organization, 2015), exerting enormous burdens on individuals and on healthcare systems (World Health Organization, 2015), especially given that there is currently no cure for T2D. Diabetes is a risk factor for a number of disabling and even life-threatening complications over the long-term. For example, one of the major long-term complications of T2D is an increased risk for developing Alzheimer's disease (AD) (Luchsinger and Gustafson, 2009; Muller et al., 2007; Vagelatos and Eslick, 2013). AD is the most common form of age-related dementia, accounting for up to 80% of dementia cases (Alzheimer's Association, 2015); an estimated 44.4 million people worldwide suffer from AD and dementia (Alzheimer's Disease International, 2013). Similar to T2D, AD exerts an enormous burden on individual patients and healthcare systems, and there is currently no cure for AD. Extensive epidemiological, clinical, and experimental evidence strongly suggest a causative role of diabetes in the onset and progression of AD-type dementia. The National Diabetes Health Fact Sheet indicates that approximately 8.3% of Americans have diabetes, and it is estimated that approximately 30% of Americans over the age of 65 affected by AD have co-morbidity with at least one serious medical condition associated with diabetes. A recent systematic meta-analysis of 15 epidemiologic studies suggests that patients with T2D have an elevated relative risk ratio of 1.57 for developing AD (Vagelatos and Eslick, 2013). Specific mechanistic interactions connecting diabetes and AD remain unknown. There is also no information on why certain subpopulations of diabetic individuals develop AD or how to identify at-risk individuals in order to target them for early, secondary preventive interventions. Mounting evidence suggests that AD dementia can be traced back to pathological conditions, such as T2D, that are initiated several decades before clinical AD onset. Since T2D is one of the potentially modifiable risk factors for AD (Luchsinger and Gustafson, 2009; Muller et al., 2007; Vagelatos and Eslick, 2013), interventions targeting T2D phenotypes prior to the onset of AD dementia represent a potentially effective secondary preventive strategy to help reduce the prevalence of AD.

Both T2D and AD are complex diseases, each involving multiple etiologic contributing factors (Gautrin and Gauthier, 1989; Henriksen et al., 2011; Jiang et al., 2013; Morris et al., 2014; Onso-Magdalena et al., 2011; Raciti et al., 2015). Among these, genetic predisposition factors are known to play important roles in both T2D and AD (Chouraki and Seshadri, 2014; Prasad and Groop, 2015; Raciti et al., 2015; Tanzi, 2012). We hypothesize that T2D may share common underlying genetic etiologies with AD, and that the presence of these shared T2D/AD genetic etiologies in a subset of individuals may contribute to the development of T2D in these individuals, as well as the development of AD over the long-term. Recent applications of Genome-Wide Association Studies (GWAS) have led to the identification of genetic variants, particularly single nucleotide polymorphisms (SNPs), for a number of complex diseases, including schizophrenia and cardiovascular diseases (Kendler, 2015;

Schunkert et al., 2011). Using GWAS methodologies, the present study investigated whether T2D and AD share common genetic etiological factors and, if so, dissected the potential impacts of these genetic factors on cellular/molecular mechanisms that may contribute to the development of T2D and AD. Outcomes from our studies provide a better understanding and will allow for improved identification of T2D subjects at genetic risk to develop AD. This information is of paramount interest in order to redirect efforts for potential therapeutic interventions in a certain T2D subpopulation, and is also a fundamental aspect in the long-term management of T2D.

2. Materials and methods

2.1. Identification of SNPs associated with risk for either T2D or AD

In order to identify the SNPs that are associated with risk for T2D, we retrieved the meta-GWAS statistics from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium study (Morris et al., 2012), which was generated from a study cohort of 34,840 T2D and 114,981 non-T2D control cases that are overwhelmingly of European descent. These T2D summary statistics provide a distribution frequency of over 2 million SNPs (imputed based on HapMap reference), among which 34,840 are T2D and 114,981 are non-T2D control cases. A meta-analysis was performed in order to join multiple individual GWAS cohorts and test individual SNPs for association with T2D.

In order to identify the SNPs that are associated with risk for AD, we retrieved meta-GWAS statistics from the International Genomics of Alzheimer's Project (IGAP) (Lambert et al., 2013) study that was generated from over 7 million directly genotyped or imputed SNPs (1000 Genome reference), for 17,008 AD cases and 37,154 non-AD control cases of European descent. Moreover, we obtained summary statistics (from the IGAP database) for 11,632 SNPs that were genotyped and tested for association with AD in an independent cohort of 8,572 AD cases and 11,312 non-AD control cases. IGAP conducted a meta-analysis combining the results of individual cohorts in order to test individual SNPs for their association with AD.

Multiple cutoff p-value criteria for the identification of significant SNP associations were considered in our analyses. For a given SNP, the allele that showed the higher frequency in cases (T2D or AD) than in controls (non-T2D or non-AD) was denoted as the risk allele.

2.2. Identification of SNPs associated with risk for both T2D and AD

The effect size attributable to individual genetic variants for a given complex disorder is typically modest, suggesting that individual genetic variants may only explain a very small amount of the genetic risk and heritability of complex disorders (Manolio and Collins, 2007). Therefore, genetic contributions to complex conditions such as T2D and AD are likely derived from a large number of genetic causal variants, each contributing a small genetic risk. Thus, we chose a "relaxed" cutoff genetic association p-value of 1E–2 as a criterion for identifying SNPs that are associated with risk for T2D or for AD in order to more comprehensively capture SNPs with small effect sizes. We then overlapped the two listings of SNPs associated with risk for T2D or AD and identified a subset of shared T2D/AD SNPs found in both listings, which are associated with risk for both T2D and AD. For each of the shared SNPs, we identified the specific SNP allele that is associated with risk for T2D and the specific SNP allele that is associated with risk for AD. We then further subdivided the shared T2D/AD SNPs into 2 subcategories: shared SNPs with "consistent risk alleles," such that the risk allele for AD is also the risk allele for T2D, and T2D/AD shared SNPs with "divergent risk alleles," such that the risk allele for T2D is different from the risk allele for AD. For a given shared T2D/AD SNP characterized by a consistent risk allele, the molecular process(es)/pathway(s) associated with the risk allele variant could be a common pathogenic factor(s) for both T2D and AD. Thus, the presence of this shared, consistent risk allele among T2D cases, could contribute to the risk for subsequent development of AD. In contrast, for a given shared T2D/AD SNP that is characterized by a divergent risk allele, the presence of the T2D risk allele variant could contribute to risk for the development of T2D but may not necessarily contribute to AD, or vice versa.

In overlap analysis, we partitioned the SNPs that were studied in both AD and T2D GWAS into four bins: (1) SNPs passed GWAS p-value cutoff in both diseases; (2) SNPs passed GWAS p-value cutoff in AD but not T2D; (3) SNPs passed GWAS p-value cutoff in T2D but not AD; (4) SNPs passed GWAS p-value cutoff in neither T2D nor AD. We then computed overlap OR and p-value (hypergeometric test) based on the contingency table formed by the four bins. Among the shared AD-T2D SNPs, we computed the p-value of risk allele consistency. Under the null hypothesis (H_o), the ratio of SNPs of consistent allele among all AD-T2D shared SNPs was 0.5. For each of the shared AD-T2D SNPs, when the observed consistent allele ratio deviated from H_o, the p-value was computed using binominal distribution.

2.3. GWAS meta-analysis from additional complex diseases

CARDIoGRAMplusC4D contains summary data from 2 large meta-analyses of coronary artery disease (CAD): (1) CARDIoGRAM (Coronary ARtery DIsease Genome wide Replication and Meta-analysis), a meta-analysis of 22 GWAS studies of European descent imputed to HapMap 2 involving 22,233 cases and 64,762 controls (Schunkert et al., 2011), and (2) C4D (The Coronary Artery Disease Genetics consortium), a meta-analysis of GWAS studies of European and South Asian descent involving 15,420 cases and 15,062 controls (Coronary Artery Disease (C4D) Genetics Consortium, 2011).

2.4. Expression quantitative trait loci (eQTL) of disease-relevant tissues

Potential influences of individual SNP genetic variants on gene expression (*i.e.*, on the genes that might be modulated by individual SNP genetic variants and/or on the direction/magnitude by which specific SNP variants may

affect gene expression) were derived primarily from available published expression quantitative trait loci (eQTL) databases. In order to dissect the potential impacts of individual SNPs on T2D etiology, we used a published eQTL database with genome-wide SNP and RNA expression information from multiple peripheral tissues that are most relevant to diabetes: liver (n = 568), omental adipose (n = 675), and subcutaneous adipose (n = 611) (Greenawalt et al., 2011).

In order to dissect the potential impacts of individual SNPs on AD etiology, we used a published eQTL database with genome-wide SNP and RNA expression information from multiple brain tissues: prefrontal cortex (pfc, n = 583), visual cortex (vc, n = 409), and cerebellum (cr, n = 496) (Podtelezhnikov et al., 2011; Zhang et al., 2013). In addition, we performed genotype imputation (1000 genome reference following MaCH software pipeline (Howie et al., 2012) and eQTL discovery). The eQTLs were quantified at 10% false discovery rate (FDR) and served as an empirical bridge between DNA polymorphisms (*e.g.*, SNPs) and transcription levels. Cis-eQTLs were used when the distance between the SNP and genes under influence were within 500 kb, and such SNPs were denoted as cis-eSNP. Trans-eQTLs were used when the distance between the SNP and gene was greater than 500 kb or were located on different chromosomes, and such SNPs were denoted as trans-eSNP. Significant eSNP is defined as the SNP-expression association that passes 10% FDR in at least one tissue. The ENCODE (Encyclopedia of DNA Elements) database was downloaded from regulomeDB (Boyle et al., 2012).

2.5. Annotation of SNPs using the an annotate variation (ANNOVAR) software

The ANNOVAR software (Wang et al., 2010) was used to annotate all variants. Functional consequences of variants were evaluated by the SIFT (Ng and Henikoff, 2003) and PolyPhen-2 (Adzhubei et al., 2010) softwares. SIFT predicts whether an amino acid substitution affects protein function based on the degree of conservation of the amino acid residues. PolyPhen-2 predicts the possible impact of amino acid substitution on the structure and function of a human protein based on physicochemical properties of the amino acids involved.

2.6. Disease Association Protein–Protein Link Evaluator (DAPPLE) analysis

The hypothesis behind DAPPLE is that causal genetic variants affect common mechanisms, and that these mechanisms can be inferred by looking for physical connections between proteins encoded in disease-associated regions (Rossin et al., 2011). Genes generated from ANNOVAR analysis were used as input to the analysis. DAPPLE constructed direct and indirect interaction networks from the input genes, assessed statistical significance of network connectivity parameters using permutation-based methods, and produced an expanded set of genes most likely to be associated with the input genes in protein–protein interaction networks.

2.7. Gene ontology and KEGG pathway enrichment analysis

KEGG pathway enrichment analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7, a tool that is able to identify the functional categories and biological processes which are most represented within a list of genes (Huang et al., 2009). Gene lists derived from shared T2D/AD SNPs with consistent risk alleles were used as input of the DAVID analysis. A program (written in R) was developed that allows execution of DAVID in a batch processing manner. The "first round" of DAVID analysis was performed using the initial ANOVAR gene lists derived from shared T2D/AD SNPs with consistent alleles. The "second round" of DAVID analysis ("real run") was performed using the initial gene list plus the gene list produced by DAPPLE.

3. Results

3.1. Identification of SNPs associated with either risk for T2D or AD

Starting with independent DIAGRAM and IGAP meta-GWAS result statistics, we used multiple GWAS genetic association p-values (ranging from 1E–8 to 1E–2) as cutoff criteria and identified SNPs that are associated with AD or T2D for specific GWAS p-value thresholds (Table 1). There is a general consensus among GWAS studies that a p-value less than 5E–8 corresponds to genome-wide significance (Stranger et al., 2011). Using a GWAS p-value threshold of 1E–8, we identified 661 and 356 SNPs that meet the generally accepted criterion for GWAS significance for association with T2D and AD, respectively (Table 1).

Identifying the genes influenced by T2D- or AD-associated SNPs is a key step toward elucidating the functional impacts of these SNPs in health and in disease. Thus, we employed large eQTL datasets of relevant tissues to empirically identify genes whose expression levels were influenced by the AD or the T2D SNPs. Our eQTL analyses were conducted using tissues relevant to T2D (*e.g.*, adipose and liver) and AD (brain); eQTLs were calibrated at 10% FDR to control for multiple testing.

Among the 661 T2D SNPs we identified at a GWAS threshold selection p-value criterion of 1E–8, we found 566

Table 1
Identification of SNPs associated with risk for T2D or AD.

GWAS p-value threshold	Number of SNPS	
	T2D	AD
1.0E–08	661	356
1.0E–07	840	448
1.0E–06	1,087	755
1.0E–05	1,516	1,296
1.0E–04	2,758	2,357
1.0E–03	8,265	6,140
1.0E–02	42,200	35,473
1.0E–01	298,496	272,746

Meta-GWAS result statistics from DIAGRAM (T2D) and IGAP (AD) were filtered using multiple GWAS threshold p-value criteria, ranging from p-value of 1E–8 to 1E–1. Shown are the total numbers of SNPs that we identified for each threshold criteria, which are associated with risk for T2D or AD.

Table 2
Genes associated with GWAS significant T2D SNPs.

(A) Genes known to associate with T2D						(B) Genes not currently associated with T2D	
Gene	No. of SNPs	Gene	No. of SNPs	Gene	No. of SNPs	Gene	No. of SNPs
CDKAL1	98	KCNQ1	10	KCNJ11	2	KIF11	16
FTO	86	HNF1B	6	TLE1	1	Y_RNA	5
TCF7L2	66	IDE	6	ABCC8	1	SNRPGP16	4
THADA	63	SLC30A8	6	COBLL1	1	AC068138.1	3
WFS1	51	CDKN2B	4	HHEX	1	ZBED3-AS1	2
IGF2BP2	43	DGKB	4	UBE2E2	1	CTD-2021H9.2	2
JAZF1	16	ARAP1	3	MTNR1B	1	RPSAP52	2
ADCY5	15	IGF2BP2	3	PROX1	1	AC022431.2	1
PPARG	11	ANK1	2	SUGP1	1	AL161652.1	1
ADAMTS9-AS2	11	ZMIZ1	2			VPS33B	1
TCF7L2	10	PPARG	2			LOC646736	1

SNPs that are associated with risk for T2D at a p-value cut-off threshold criteria of 1E–8 were analyzed using a large eQTL dataset of brain tissues to empirically identify genes whose expression levels were influenced by the AD SNPs. Presented are cis-eSNPS annotations based on cis-qQTL analysis. Shown are genes whose expression in the peripheral tissues (*e.g.*, omental and subcutaneous adipose and liver tissues) are associated with the GWAS significant T2D SNPs. (A,B) Segregation of annotated genes to those that are known to associate with T2D (A), and those are not currently associated with T2D (B).

SNPs are associated with the expression regulation of 42 known genes; the remaining 95 SNPs are not associated with gene expression regulations. The 42 genes which could be modulated by T2D SNPs are shown in Table 2. Most of these genes are modulated by multiple T2D SNPs (Table 2A and 2B). Moreover, among the 42 genes that are associated with the T2D SNPs, 31 genes have already been related to T2D (Table 2A), while the published literature has not yet associated the remaining 11 genes with T2D (Table 2B).

Similarly, at a GWAS threshold selection p-value criterion of 1E–8, we identified 356 AD SNPs. Among these, 267 SNPs are associated with gene expression regulation of 50 known genes, and 80 SNPs are not associated with gene expression regulation. Similarly to our observation with the T2D SNPs, most of the genes we found associated with the AD SNPs are also modulated by multiple AD-SNPs (Table 3A and 3B). Among the 50 genes that are associated with the AD-SNPs, 29 genes have been related to AD (Table 3A) and the remaining 21 genes have not yet been associated with T2D (Table 3B).

3.2. Overlap of T2D and AD GWAS SNPs

We hypothesized that T2D and AD share common genetic etiology. Given that detailed results from powerful meta-GWAS studies with large sample sizes on T2D and AD are publicly available, our hypothesis could be directly tested by examining whether the overlap of significant GWAS SNPs for T2D and AD are greater than random chance. Therefore, we retrieved GWAS summary statistics (*e.g.*, association p-values and directions) for T2D from the DIAGRAM consortium study and for AD from the IGAP study. We then filtered the summary statistics using multiple GWAS genetic association p-values, ranging from 1E–8 to 1E–1, as cutoff selection criteria and identified, for each of the p-value selection criterion, SNPs that are associated with risk for T2D or for AD (Table 1). Among the ~2 million tested in both T2D and AD GWAS studies, the proportion of associated SNPs at any given p-value threshold was greater than alpha ($p = 0.05$) level, suggesting genetic signals underlying these 2 diseases. For example, at p-value ≤1E–3 threshold, 8,265 SNPs

Table 3
Genes associated with GWAS significant AD SNPs.

(A) Genes known to associate with AD						(B) Genes not currently associated with AD			
Gene	No. of SNPs	Gene	No. of SNPs	Gene	No. of SNPs	Gene	No. of SNPs	Gene	No. of SNPs
PICALM	31	APOE	5	GAB2	1	CLPTM1	13	NYAP1	1
MS4A4A/4E/6A/6E	28	SORL1	5	GLIS3	1	AP001257.1	6	ANKRD55	1
CD2AP	24	APOC1	3	MTHFD1L	1	BCAM	5	APOC1P1	1
TOMM40	23	BCL3	3	PDE7B	1	GULOP	3	CEACAM16	1
MS4A2	15	CLU	3	SLC24A4	1	HMHA1	3	CR2	1
PVRL2	13	PTK2B	3	SPON1	1	RNU6-560P	3	EXOC3L2, MARK4	1
APOC2/APOC4	12	ABCA7	2	ZCWPW1	1	XXbac-BPG254F23.7	3	NYAP1	1
MARK4	11	PVR	2			CEACAM19	3	PILRA	1
BIN1	7	CD33	1			AL355353.1	2	RNU6-560P	1
CR1	7	CTNNA2	1			GPR111	2	snoU13	1
EPHA1/EPHA1-AS1	7	FERMT2	1			snoZ6	2		

SNPs that are associated with risk for AD at a p-value cut-off threshold criteria of 1E–8 were analyzed using a large eQTL dataset of brain tissues to empirically identify genes whose expression levels were influenced by the T2D SNPs. Presented are cis-eSNPS annotations based on cis-qQTL analysis. Shown are genes whose expressions in the brain tissues (*e.g.*, frontal cortex, visual cortex and cerebellum) are associated with the GWAS significant AD SNPs. (A,B) Segregation of annotated genes to those that are known to associate with AD (A), and those are not currently associated with AD (B). (A,B) The "no. of SNPs" reflects the number of SNPs that are associated with expression levels of the specific genes.

Table 4
Overlap of GWAS signals for AD and T2D.

(A)

Comparison	GWAS p-value threshold	Overlap OR	Overlap p-value	Number of overlap SNPs	N of overlap SNPs with consistent risk allele	% SNPs with consistent risk allele	p-Value of risk allele consistency
AD *vs.* T2D	1.00E–06	2.87	2.95E–01	1	0	0	1.00E+00
	1.00E–05	4.8	1.05E–02	4	0	0	1.25E–01
	1.00E–04	9.94	4.45E–18	27	2	7.41	5.65E–06
	1.00E–03	3.19	1.02E–15	68	7	10.29	7.39E–12
	1.00E–02	1.48	6.93E–28	927	395	42.61	7.66E–06
	1.00E–01	1.04	1.70E–10	35,719	18,085	50.63	1.73E–02

(B)

GWAS p-value threshold	Consistent risk allele			Divergent risk allele		
	Number of overlap SNP	Overlap OR	Overlap p-value	Number of overlap SNP	Overlap OR	Overlap p-value
1.00E–06	0	–	1	1	8.25	1.15E–01
1.00E–05	0	–	1	4	13.18	2.86E–04
1.00E–04	2	1.33	5.65E–06	25	21.68	1.31E–24
1.00E–03	7	0.46	7.39E–12	61	5.82	2.72E–26
1.00E–02	395	1.2	7.66E–06	532	1.72	1.43E–29

(A) Meta-GWAS result statistics were retrieved from IGAP (AD study) and Diagram (T2D study) databases, and filtered by meta-analysis p-values to identify shared T2D/AD SNPs that are found in both listings. The following information is presented: overlap odds ratio, Fisher test p-value of overlapping, the number of overlapping SNPs, the number of SNPs with the same risk allele in disease GWAS, the percentage of overlapping SNPs with the same risk allele in disease GWAS over total number of overlapping SNPs, the binomial test p-value of consistent direction among overlapping significant SNPs. (B) We stratified the SNPs tested in both AD and T2D meta-GWAS by the risk allele consistency regardless of associated p-value. A SNP where the same allele was associated with a higher risk for both AD and T2D was termed consistent risk allele SNP. Otherwise, they are referred to as divergent risk allele SNP. Shown are the number of overlapping SNPs with consistent (or divergent) risk alleles, corresponding overlap odds ratio, and p-value of overlapping.

and 6,140 SNPs were associated with T2D and AD, respectively (Table 1).

For each of the p-value selection criteria, we overlapped the identified T2D and AD GWAS SNPs in order to identify shared T2D/AD SNPs that are associated with risk for both T2D and AD (Table 4A). For example, at p-value ≤1E–3 threshold, 68 SNPs were associated with both diseases, which is significantly larger than random chance (overlap p-value of 1.02E–15 and overlap odds ratio = 3.19). Our observation that the overlapping of T2D and AD GWAS SNPs are significant across multiple p-value selection criteria suggests that T2D and AD may share genetic etiological risk factors. In contrast to our observation, previous investigations demonstrated that two large disease GWAS may show no significant overlap of shared SNPs. For example, GWAS from obesity and age of menopause did not show a significant overlap (Locke et al., 2015). Thus, our observed statistical significance for the presence of shared SNPs that are associated with both T2D and AD is not likely to be a chance finding of simply overlapping GWAS of unrelated diseases.

3.3. Association direction of shared T2D and AD SNPs

The shared T2D/AD SNPs can be segregated into shared SNPs with consistent risk alleles (the risk allele for T2D is also the risk allele for AD) and those with divergent risk alleles (the risk allele for T2D is different from the risk allele for AD). Our working hypothesis is that the presence of shared T2D/AD SNPs with consistent risk alleles would contribute to the correlation between these 2 diseases' incidence. Thus, we stratified the shared T2D/AD SNPs into the consistent risk allele SNP and divergent risk allele SNP subcategories. Regardless of GWAS test p-values criteria, if one allele of an SNP was associated with higher risk of both AD and T2D, the SNP was categorized as a consistent risk allele SNP; otherwise, the SNP was categorized as a divergent risk allele SNP.

At a GWAS p-value ≤1E–2, we found that 395 shared SNPs have the same risk allele for both AD and T2D (overlap p-value of 7.66E–6, overlap OR = 1.2, Table 4B). Our observation is consistent with a common genetic basis underlying both diseases. Interestingly, at a GWAS p-value ≤1E–2, we observed 532 shared SNPs that have divergent risk alleles for AD versus T2D (overlap p-value of 1.43E–29, overlap OR = 1.72, Table 4B). This suggests that some of the T2D/AD shared SNPs may contribute to one of the diseases without necessarily contributing to the other.

Our observation that shared T2D/AD SNPs can be subdivided into shared SNPs with consistent risk alleles versus shared SNPs with divergent risk alleles can also be seen in other diseases. For example, consistent with the strong linkage between T2D and coronary artery disease (CAD) (Wang et al., 2014), we observed an extensive overlap between T2D and CAD GWAS signals (Table 5). For example, at GWAS p-value ≤1E–4 threshold, 59 SNPs were associated with both T2D and CAD (p-value of 7.97E–65 and the overlap OR = 31.39). We found that 57 of the shared T2D/CAD SNPs are characterized by consistent risk alleles (96.61; overlap p-value of 6.14E–15, Table 5). Our observation suggests that the investigation of shared GWAS risk SNPs should be further delineated into the association direction of the shared SNPs.

Table 5
Overlap of GWAS signals for T2D *vs.* CAD.

Comparison	GWAS p-value threshold	Overlap OR	Overlap p-value	No. of overlap SNPs	No. of overlap SNPs with consistent risk allele	% SNPs with consistent risk allele	p-Value of risk allele consistency
T2D *vs.* CAD	1.E–08	90.42	6.21E–06	3	3	100	2.50E–01
	1.E–07	330.39	1.29E–32	15	15	100	6.10E–05
	1.E–06	254.24	1.41E–48	24	24	100	1.19E–07
	1.E–05	102.54	4.50E–44	27	27	100	1.49E–08
	1.E–04	31.39	7.97E–65	59	57	96.61	6.14E–15
	1.E–03	8.41	9.02E–117	215	188	87.44	7.00E–31
	1.E–02	2.11	5.02E–157	1,695	1446	85.31	5.75E–205
	1.E–01	1.15	1.07E–138	47,327	31,648	66.87	9.88E–324

Meta-GWAS result statistics retrieved from DIAGRAM (T2D) and cardiogramplusC4D (CAD) databases were filtered by multiple meta-analysis p-values. Filtered SNPs were overlapped across T2D and CAD. The T2D *vs.* CAD comparison shows, across multiple GWAS p-values, the total number of overlapping shared T2D/CAD SNPs, the corresponding overlapping odds ratio and Fisher test p-value of overlapping, which revealed overlapping of the shared SNPs was greater than random chance. Also shown is the number of overlapping T2D/CAD SNPs with consistent risk allele, percentage of overlapping SNPs with the same risk allele in disease GWAS over total number of overlapping SNPs, and binomial test p-value of consistent direction among overlapping significant SNPs.

3.4. Genes influenced by the shared T2D/AD SNPs

Shared T2D/AD SNPs may undergo complicated pathways and lead to disease risk. Identifying the genes influenced by such shared SNPs would be the key step elucidating the SNPs' function and pathogenic pathways on T2D and/or AD. To investigate potential functional impacts of T2D/AD shared SNPs, we analyzed the 927 shared T2D/AD SNPs we identified using a "relax" p-value threshold criterion of 1E–2 for GWAS genetic association with T2D and AD (Table 4). This is because the effect size attributable to individual genetic variants for a given complex disorder is typically modest, suggesting that individual genetic variants may only explain a very small amount of the genetic risk and heritability of complex disorders (Manolio and Collins, 2007). Genetic contributions to complex conditions, such as T2D and AD, are likely derived from a large number of genetic causal variants, each contributing a small genetic risk. Thus, our choice of using a T2D/AD shared SNPs generated from a relax cutoff genetic association p-value of 1E–2 will comprehensively capture SNPs with small effect sizes on T2D and AD.

Among the 927 shared T2D/AD SNPs that we identified, 395 are characterized by having consistent risk alleles and 532 are characterized by having divergent risk alleles (Table 4). We annotated individual shared T2D/AD SNPs characterized by consistent and divergent risk alleles using the ANNOVAR (Annotated Variation) software (Wang et al., 2010). Shared T2D/AD SNPs with consistent or divergent risk alleles are listed in Supplementary Tables S1 and S2, respectively. Also shown for each of the shared T2D/AD SNPs are the corresponding p-values associated with risk for T2D and AD, the identity of risk alleles for T2D and/or AD, and the resultant gene annotations from ANNOVAR analyses for each of shared T2D/AD SNPs (Supplementary Tables S1 and S2). Since causal genetic variants affecting common mechanisms can be inferred by examining physical connections between proteins encoded in disease-associated region (Rossin et al., 2011), we used the DAPPLE (Disease Association Protein–Protein Link Evaluator) software (Rossin et al., 2011) to identify proteins that are known to physically interact with each of the annotations generated from ANNOVAR from shared T2D/AD SNPs. Results from the DAPPLE analysis produced an additional 190 genes that are functionally connected to T2D/AD with consistent risk alleles (Supplementary Table S3) and an additional 385 genes that are functionally connected to shared T2D/AD SNPs with divergent risk alleles (Supplementary Table S4).

3.5. Functional annotation of the genes influenced by share T2D and AD GWAS signals

Based on our working hypothesis that individuals with the presence of these shared T2D/AD genetic etiologies may contribute to the development of T2D in these individuals, as well as the development of AD over the long-term, we investigated the potential functional impacts of T2D/AD SNPs with consistent risk alleles. Gene annotations for each of the shared T2D/AD SNPs with consistent risk alleles were generated using ANNOVAR and DAPPLE. The combined gene annotation from ANNOVAR and DAPPLE were assessed for functional pathway enrichment by assessing the corresponding annotated genes for gene enrichments among known KEGG functional pathways. To evaluate the significance of the pathways generated in the second round "real run" DAVID analysis, 20 "decoy runs" of DAVID were performed, using the initial gene list plus equal numbers (to that of "additional genes") of randomly selected human genes. The lowest p-value obtained in the 20 "decoy runs" was 1.29E–3, and was chosen as the Benjamini-corrected p-value cut-off to determine "significant" KEGG pathway enrichment.

We found significant enrichment of the annotated genes in 14 KEGG pathways (Table 6). For each of these KEGG pathways, information regarding the total number of genes in the pathway, the number (and the name) of annotated genes found in the pathway, fold enrichment and the Benjamini-corrected p-value for enrichment are shown in Table 5. Notably, we observed that 5 of the enriched KEGG pathways listed in Table 6 pertain to immune responses (*e.g.*, Fc gamma R-mediated phagocytosis and chemokine signaling), cell signaling (*e.g.*, MAPK and Wnt signaling) and

Table 6
KEGG functional enrichment analysis.

Term	Total pathway size	No. of genes modulated by the shared T2D/AD SNPs	Fold enrichment	Benjamini-corrected p-value	Genes
hsa04540: Gap junction	89	20	8.4	8.2E–11	PRKCA, EGFR, GNAI3, GNAI2, GRB2, ADCY8, GNAI1, GNA11, ADCY5, PRKCG, SRC, PRKX, PRKCB, PRKACG, TUBB, TJP1, PRKACA, PRKACB, TUBA1A, HTR2A
hsa04916: Melanogenesis	99	19	7.2	2.0E–09	PRKCA, GNAI3, GNAI2, ADCY8, GNAI1, ADCY5, CREBBP, PRKCG, TCF7L2, PRKX, CTNNB1, PRKCB, PRKACG, EP300, CAMK2D, CALM3, PRKACA, PRKACB, CAMK2A, CALM2, CALM1
hsa04720: Long-term potentiation	68	15	8.2	4.3E–08	PRKCA, ADCY8, CREBBP, PRKCG, PRKX, PRKCB, PRKACG, EP300, CAMK2D, CALM3, PRKACA, PRKACB, PPP3CA, CACNA1C, CAMK2A, CALM2, CALM1
hsa04912: GnRH signaling pathway	98	17	6.5	8.1E–08	PRKCA, EGFR, GRB2, ADCY8, GNA11, ADCY5, SRC, PRKX, PRKCB, PRKACG, PTK2B, CAMK2D, CALM3, PRKACA, PRKACB, CACNA1C, CAMK2A, CALM2, CALM1
hsa05414: Dilated cardiomyopathy	92	14	5.7	9.3E–06	ACTB, ACTC1, ADCY8, ADCY5, CACNB1, TPM2, PRKX, PRKACG, ACTG1, ITGA8, RYR2, PRKACA, PRKACB, CACNA1C
hsa04666: Fc gamma R-mediated phagocytosis	95	14	5.5	1.2E–05	PRKCA, DNM3, VAV3, PRKCG, ARPC5, VAV2, TTLL3, PRKCB, ARPC1A, ARPC3, ARPC2, SCIN, PLA2G4F, DNM2
hsa04020: Calcium signaling pathway	176	18	3.8	3.1E–05	PRKCA, EGFR, ADCY8, GNA11, PRKCG, PRKX, PRKCB, PRKACG, PTK2B, CAMK2D, CALM3, RYR2, PRKACA, PRKACB, PPP3CA, CACNA1C, CAMK2A, CALM2, CALM1, HTR2A
hsa04062: Chemokine signaling pathway	187	18	3.6	6.5E–05	GNAI3, VAV3, GNAI2, GRB2, ADCY8, GNAI1, ADCY5, PF4V1, VAV2, PRKX, PRKCB, PRKACG, PTK2B, ARRB1, TIAM1, JAK2, PRKACA, PRKACB
hsa05110: Vibrio cholerae infection	56	10	6.7	1.0E–04	PRKCA, ACTG1, PRKACG, ACTB, TJP1, PRKCG, PRKACA, PRKACB, PRKX, PRKCB
hsa04310: Wnt signaling pathway	151	15	3.7	2.8E–04	PRKCA, TBL1XR1, CREBBP, PRKCG, TCF7L2, PRKX, PRKCB, CTNNB1, PRKACG, EP300, CAMK2D, PRKACA, PRKACB, PPP3CA, CAMK2A
hsa04010: MAPK signaling pathway	267	20	2.8	4.5E–04	PRKCA, EGFR, TAOK2, FGF14, GRB2, CACNB1, PRKCG, SRF, DAXX, FLNA, PRKX, PRKCB, PRKACG, ARRB1, PRKACA, PPP3CA, PRKACB, CACNA1C, HSPA8, PPP5C
hsa04914: Progesterone-mediated oocyte maturation	86	11	4.8	5.1E–04	HSP90AB1, PRKACG, GNAI3, GNAI2, GNAI1, ADCY8, ADCY5, PRKACA, PRKACB, CDK2, PRKX
hsa04114: Oocyte meiosis	110	12	4.1	8.4E–04	AR, ADCY8, ADCY5, PRKX, CDK2, PRKACG, CAMK2D, CALM3, PRKACA, PRKACB, PPP3CA, CAMK2A, CALM2, CALM1
hsa04270: Vascular smooth muscle contraction	112	12	4	8.9E–04	PRKCA, ADCY8, ADCY5, GNA11, PRKCG, PRKX, PRKCB, PRKACG, CALM3, PRKACA, PRKACB, CACNA1C, CALM2, CALM1

The analysis was conducted using combined gene annotations from ANNOVAR and DAPPLE that are generated from shared T2D/AD SNPs with consistent risk alleles. The KEGG pathway analysis was performed using the DAVID tool (http://david.abcc.ncifcrf.gov/). DAVID determines the significance of a pathway by Fisher's exact test, which directly takes into account the pathway size. Fisher's exact test has 4 build-in parameters: total number of genes in the genome, total number of genes in the input gene set, the number of genes in the genome belonging to a specific KEGG pathway, and the number of genes in the input gene set that belong to the specific KEGG pathway. Moreover, we adopted a decoy run-based procedure to determine the truly significant pathways: 20 decoy runs were executed in addition to the "real" run. This procedure fully eliminates any remaining unwanted confounding effects, due to pathway size or other conceivable factors. Listed are 14 KEGG pathways with significant enrichments (Benjamini-corrected p-value ≤1.29E–3) of the annotated genes. Shown are the total number of genes in the pathway, the number (and the name) of annotated genes found in the pathway, fold enrichment and Benjamini-corrected p-value for enrichment.

neuronal plasticity (*e.g.*, long-term potentiation), and dysfunction of these processes are known to contribute to both T2D and AD (Balietti et al., 2012; Bordonaro, 2009; Cruz et al., 2013; Evans et al., 2002; Kim and Choi, 2010; Lee et al., 2010; Rios et al., 2014; Wang et al., 2013).

4. Discussion

In this study, we employed a systems biology approach in order to examine shared genetic risk factors and functional categories between AD and T2D. We integrated

large-scale GWAS, pathway and gene ontology data, eQTL, co-expression networks, and regulatory elements.

We used multiple GWAS genetic association p-values (ranging from 1E–8 to 1E–2) as cutoff criteria and identified SNPs that are associated with AD or T2D for specific GWAS p-value thresholds. An interesting finding from this analysis is the identification of 661 SNPs that meet the generally accepted criterion (GWAS p-value threshold of 1E–8) of GWAS significance for association with risk for T2D. We found these SNPs are associated with the expression regulation of 31 genes that have already been associated with T2D and 11 genes that have not yet been associated with T2D in the published literature. Similarly, we found 356 SNPs that meets the generally accepted criterion (GWAS p-value threshold of 1E–8) of GWAS significance for association with risk for AD. These AD-associated SNPs are associated with the expression regulation of 29 genes that have been related to AD and 21 genes that have not yet been associated with T2D. Similarly, at a GWAS threshold selection p-value criterion of 1E–8, we identified 356 AD SNPs. Among these, 267 SNPs are associated with gene expression regulation of 50 known genes, and 80 SNPs are not associated with gene expression regulation. Similar to our observation with the T2D SNPs, most of the genes we found associated with the AD SNPs are also modulated by multiple AD-SNPs. Among the 50 genes that are associated with the AD-SNPs, 29 genes have been related to AD and 21 genes have not yet been associated with T2D (Table 3B). Collectively, our genetic association evidence is consistent with published information regarding the genetics and/or molecular pathogenic etiologies underlying T2D and AD. Moreover, our observation revealing additional genes not currently associated with T2D or AD could serve as novel molecular targets for future investigations.

Comparing SNPs that are associated with risk for T2D with SNPs that are associated with risk for AD, we observed significant overlapping of T2D and AD GWAS SNPs across multiple p-value selection criteria (Table 4). Our observation provides the first evidence suggesting that T2D and AD may share genetic etiological risk factors. In contrast to our observation, previous investigations demonstrated that two large disease GWAS may show no significant overlap of shared SNPs. For example, GWAS results on obesity and age of menopause did not show a significant overlap (Locke et al., 2015). Thus, our observed statistical significance for the presence of shared SNPs that are associated with both T2D and AD is likely not a chance finding of simply overlapping GWAS of unrelated diseases.

We note, however, that the shared T2D/AD SNPs can be stratified into shared SNPs with consistent risk alleles or with divergent risk alleles (Table 4, Supplementary Tables S1 and S2). Shared SNPs with consistent risk alleles would represent genetic etiologic factors underlying pathogenic mechanisms that are common for both T2D and AD, and that the presence of these shared T2D/AD genetic predisposition factor(s) in a subset of individuals may mechanistically contribute to the development of T2D in these individuals, as well as to the development of AD over the long-term. Consistent with this, our evidence suggests that genes that are directly and indirectly modulated by shared T2D/AD SNPs with consistent alleles are significantly enriched for multiple functional pathways pertaining to immune responses (*e.g.*, Fc gamma R-mediated phagocytosis and chemokine signaling), cell signaling (*e.g.*, MAPK and Wnt signaling), and neuronal plasticity (*e.g.*, long-term potentiation) (Table 6). Abnormal, hyper-inflammatory responses (Lee et al., 2010), activation of MAPK signaling (Evans et al., 2002; Kim and Choi, 2010), reductions in Wnt signaling (Bordonaro, 2009; Rios et al., 2014), and neuronal plasticity dysfunction (Balietti et al., 2012; Wang et al., 2013) are known to contribute to both T2D and AD pathogenesis. Thus, our observation suggests that common genetic predispositions (*e.g.*, SNPs with consistent risk alleles) in T2D and AD underlying anomalies to these pathogenic pathways could contribute to the elevated risks of subjects with T2D to eventually develop AD.

It is important to note that a large number of the shared T2D/AD GWAS SNPs we identified are characterized by divergent risk alleles in the two diseases. For individual shared T2D/AD SNPs with divergent alleles, one of the allelic forms may contribute to one of the diseases (*e.g.*, T2D), but not necessarily to the other (*e.g.*, AD), or vice versa. Thus, GWAS studies comparing two diseases aiming to identify how common genetic variants might mechanistically contribute to the two diseases not only need to identify overlapping SNPs that are associated with risks for both disease, but also need to carefully consider the risk allele consistency.

Current T2D treatments are generally designed to target pathologic processes, such as hyperglycemic, that underlie or are related to key T2D-type peripheral metabolic impairment phenotypes. Since subjects with T2D are at higher risk to develop AD (Vagelatos and Eslick, 2013), there is a general anticipation that such T2D interventions may also reduce the risk of these subjects for eventually developing AD dementia. However, recent evidence suggesting that interventions designed to modulate metabolic responses in T2D may not necessarily reduce the risk of T2D subjects to develop AD. In particular, a large clinical trial demonstrated that tight control of peripheral blood glucose does not improve cognitive (or other health) outcomes in older persons with peripheral insulin resistance (Launer et al., 2011). Thus, there is a great need to develop novel strategies to interfere with the risk of individuals with T2D to development of AD dementia. Collectively, our observations suggest that, among T2D subjects with shared SNPs having consistent risk alleles for T2D and AD as common genetic predisposition factors for both diseases, pathogenic pathways mediated by these shared SNPs may mechanistically contribute to the risk of these subjects to eventually develop AD. Our evidence provides the much needed information for the design of future novel therapeutic approaches, specifically targeting a subpopulation of T2D subjects with genetic disposition to AD, to simultaneously modulate T2D phenotypes and risk for AD dementia,

Some of the pathogenic mechanisms that are associated with T2D [*e.g.*, inflammation (De Felice and Ferreira, 2014) and reduced insulin sensitivity (Watson and Craft, 2003)] are also associated with AD. Thus, it is conceivable that intervention strategies originally developed to target T2D phenotypes might also be useful for treating AD. Interestingly, recent clinical evidence suggests that treatment with insulin, particularly via an intranasal administration protocol to promote delivery of insulin to the central nervous

system, is effective in improving cognitive functions in patients with mild to moderate AD or, in patients with amnestic mild cognitive impairment who are at high risk for developing frank AD dementia, is effective in protecting against cognitive decline (Craft et al., 2012). However, other studies with rosiglitazone and pioglitazone that are known for their insulin-sensitizing and anti-inflammatory effects failed to demonstrate a promotion of cognitive function in subjects with probable AD (Miller et al., 2011), While more studies will be necessary to investigate why certain anti-T2D strategies might (or might not) be useful for treating AD, it is conceivable that these investigations should be focused on effects of individual interventions on the appropriate subpopulation of MCI or probable AD cases. Our observation of shared T2D/AD SNPs with consistent risk alleles underlying pathogenic processes that are related to hyper-inflammatory responses, activation of MAPK signaling, reductions in Wnt signaling, and dysfunctions in neuronal plasticity provides novel genetic predisposition factors for selection of the most appropriate MCI/pre-AD subjects for clinical investigations of interventions targeting these pathogenic processes.

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Appendix: Supplementary material

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