TCF7L2 IncRNA: a link between bipolar disorder and body mass index through glucocorticoid signaling

Duan Liu1,2, Thanh Thanh Le Nguyen3,8, Huanyao Gao1, Huaiizhi Huang1,2, Daniel C. Kim5, Brenna Sharp1, Zhenqing Ye3, Jeong-Heon Lee4, Brandon J. Coombes3, Tamas Ordog1,5,6, Liewei Wang1, Joanna M. Biernacka3,7, Mark A. Frye7,8, and Richard M. Weinshilboum9

© The Author(s) 2021

Bipolar disorder (BD) and obesity are highly comorbid. We previously performed a genome-wide association study (GWAS) for BD risk accounting for the effect of body mass index (BMI), which identified a genome-wide significant single-nucleotide polymorphism (SNP) in the gene encoding the transcription factor 7 like 2 (TCF7L2). However, the molecular function of TCF7L2 in the central nervous system (CNS) and its possible role in the BD and BMI interaction remained unclear. In the present study, we demonstrated by studying human induced pluripotent stem cell (hiPSC)-derived astrocytes, cells that highly express TCF7L2 in the CNS, that the BD-BMI GWAS risk SNP is associated with glucocorticoid-dependent repression of the expression of a previously uncharacterized TCF7L2 transcript variant. That transcript is a long non-coding RNA (IncRNA-TCF7L2) that is highly expressed in the CNS but not in peripheral tissues such as the liver and pancreas that are involved in metabolism. In astrocytes, knockdown of the IncRNA-TCF7L2 resulted in decreased expression of the parent gene, TCF7L2, as well as alterations in the expression of a series of genes involved in insulin signaling and diabetes. We also studied the function of TCF7L2 in hiPSC-derived astrocytes by integrating RNA sequencing data after TCF7L2 knockdown with TCF7L2 chromatin-immunoprecipitation sequencing (ChIP-seq) data. Those studies showed that TCF7L2 directly regulated a series of BD risk genes. In summary, these results support the existence of a CNS-based mechanism underlying BD-BMI genetic risk, a mechanism based on a glucocorticoid-dependent expression quantitative trait locus that regulates the expression of a novel TCF7L2 non-coding transcript.

Molecular Psychiatry (2021) 26:7454–7464; https://doi.org/10.1038/s41380-021-01274-z

INTRODUCTION

Bipolar disorder (BD) is a psychiatric disease with significant morbidity and mortality [1]. BD is often comorbid with other disorders such as alcohol use disorder [2–4] and binge eating disorder [5]. The overall heritability of BD is between 60% and 85%, indicating that genetic factors contribute substantially to disease risk [6]. Genome-wide association studies (GWAS) have identified >60 genome-wide significant loci associated with BD risk [6–13]. Pathway enrichment analyses of BD risk genes have revealed several significant gene sets, including those that regulate insulin secretion, energy metabolism [6, 7], and corticotropin-releasing hormone (CRH) signaling [14]. Those results are consistent with clinical observations that BD is often associated with binge eating disorder [5], addiction disorders [2–4, 15] (i.e., alcohol and food), and with metabolic syndromes [16]. The prevalence of obesity in BD patients is more than twofold greater than that for the general population [16, 17]. Furthermore, obesity in BD is associated with more severe mood symptoms and worse treatment outcomes [18, 19]. The pathophysiology underlying the comorbidity of BD and obesity remains unclear.

In an attempt to gain a greater understanding of the potential contribution of genetic factors to the comorbidity of BD and obesity, we performed a GWAS to identify variants associated with body mass index (BMI)-dependent BD [20]. Even though BMI is an imperfect measure of excessive fat accumulation, it is a widely used clinical variable in studies of obesity [21, 22]. That GWAS identified a genome-wide significant single-nucleotide polymorphism (SNP) (rs12772424, P = 2.85E-08) that mapped to an intrinsic region of the TCF7L2 gene [20]. Specifically, the minor allele for the rs12772424 SNP was more strongly associated with BD as BMI increased [20]. However, this SNP was not associated with risk for either BD or BMI alone, but only showed a significant association with BD when accounting for the effect of BMI [20]. The association of this TCF7L2 SNP with BMI-dependent BD risk was later replicated in an independent cohort of BD patients [23]. TCF7L2 is a well-known risk gene for type 2 diabetes (T2D). Specifically, a different TCF7L2 SNP (rs7903146)—a SNP that is not in linkage disequilibrium (LD) with rs12772424 (r² < 0.035, D’ < 0.282) and which maps 122.2 kb distant from rs12772424—has been associated with risk for T2D in a series of studies [24–27].
The existence of two independent TCF7L2 SNPs, each of which is associated with different diseases, suggests that TCF7L2 might have different functions in T2D and in BD associated with BMI.

TCF7L2 encodes the transcription factor 7 like 2, which plays an important role in the Wnt/β-catenin signaling pathway [28], a pathway known to be involved in the pharmacotherapy of BD [29]. TCF7L2 is widely expressed across many human organs and tissues, including the brain [30]. As TCF7L2 is well recognized as a T2D risk gene, its function in T2D and glucocorticoid metabolism has been studied extensively in animal models with results that suggest a complex molecular role for this protein [31]. Specifically, Tcf7l2 dysfunction in hepatic [32–34] and pancreatic [35, 36] tissue, as well as involvement in the gut-brain axis [37] have all been thought to contribute to T2D risk. Tcf7l2 function in the central nervous system (CNS) has also been studied in rodents [38–40]. In addition to disrupted systemic glucose homeostasis, abnormalities in nervous system morphology and neurologically based behaviors such as polyphagia and hypoactivity were observed in Tcf7l2 knockout mice [41, 42]. Despite mounting evidence that TCF7L2 may link psychiatric disorders and metabolic dysregulation [20, 43], its function in human CNS cell line models, to our knowledge, has not been reported.

In the present study, we set out to characterize the molecular function of TCF7L2 in the CNS with the goal of understanding its possible role in the comorbidity of BD and obesity. These studies began with a review of human brain single-nucleus and single-cell RNA-seq data for TCF7L2, results which showed that it is most highly expressed in astrocytes. Furthermore, an examination of the genomic architecture surrounding the BD-BMI interaction GWAS identified SNP, rs12772424, showed that the variant SNP genotype created a half-palindrome glucocorticoid response element (GRE)—raising the possibility that TCF7L2 transcription might be regulated by glucocorticoid signaling. After treatment of hiPSC-derived astrocytes with dexamethasone (DEX), a potent synthetic glucocorticoid, expression levels of two TCF7L2 long non-coding RNA transcripts (lncRNA-TCF7L2) were found to be significantly upregulated. One of the lncRNA-TCF7L2 transcripts, namely “T-3”, was predominantly expressed in the human brain but not in peripheral tissues such as the liver or pancreas. As a result, after starting with an SNP that was associated with a BD subtype, the series of experiments described subsequently showed that the SNP was a pharmacogenomic expression quantitative trait locus (pQTL), i.e., an eQTL that is functional only in the presence of a drug (e.g., DEX) or endogenous hormones such as glucocorticoids [44–46]. Finally, we found that the “T-3” lncRNA-TCF7L2 influenced expression in astrocytes of both the parent gene and scores of other genes involved in both energy metabolism and BD risk. This series of studies identified and functionally characterized a TCF7L2 lncRNA and its SNP-dependent expression as a molecular mechanism related to BD-BMI interaction and, as a result, illustrates the potential importance of lncRNAs for neuropsychiatry.

MATERIALS AND METHODS

TCF7L2 transcriptional analysis in the human brain at the single-cell level

A single-nucleus RNA sequencing (snRNA-seq) data set generated by the Allen Institute that included samples across multiple human cortical areas [47] was consulted to obtain information with regard to TCF7L2 expression in CNS cells. In addition, single-cell RNA sequencing (scRNA-seq) data generated using surgically removed human cerebral cortical samples from 12 subjects [48, 49] were downloaded and combined for analysis of TCF7L2 expression. Additionally, chromatin accessibility information for the TCF7L2 gene in human brain cells was obtained from a single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) data set [50] to evaluate the transcriptional activity of TCF7L2 in clustered CNS cell types. The sources of these data sets and other key resources used in this study are listed in Supplementary Table S1. Please see the Supplementary Text for details.

Identification of the TCF7L2 transcript variants regulated by glucocorticoid signaling

The TCF7L2 rs12772424 SNP was annotated using HaploReg v4.1 [51], which indicated that the rs12772424 variant allele (A) created a half-palindromic GRE. To test the hypothesis that TCF7L2 transcription might be regulated by glucocorticoid signaling, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to quantitate RNA levels for all known TCF7L2 transcript variants in a variety of cell lines before and after treatment with the glucocorticoid receptor (GR) agonist DEX. The rs12772424 SNP effect on TCF7L2 transcriptional activity was also determined by reporter gene assay with the comparison of luciferase activities for rs12772424 SNP wild-type and variant cDNA constructs. See the Supplementary Text for details.

Characterization of TCF7L2 long non-coding RNA transcripts

Expression levels of TCF7L2 long non-coding RNA transcripts (lncRNA-TCF7L2) in the human brain and peripheral tissues as well as in human cell lines were quantified by qRT-PCR using primers targeted to their unique exon junctions. The existence of lncRNA-TCF7L2 “T-3” was confirmed by cDNA amplification of its “full length” sequence using total RNA samples prepared from human brain tissues and from hiPSC-derived astrocytes. RNA-seq was also performed with samples from hiPSC-derived astrocytes that have been transfected with ASOs to knockdown (KD) the lncRNA-TCF7L2 or with non-targeting control ASOs. Differentially expressed genes (DEGs) after KD of the lncRNA-TCF7L2 “T-3” were identified by comparison of transcriptomes of control and KD samples. Those DEGs were then used for pathway enrichment analysis using Enrichr [52] to annotate the function of the lncRNA-TCF7L2 “T-3”. See the Supplementary Text for additional details.

Identification of TCF7L2 target genes in hiPSC-derived astrocytes

RNA-seq and ChIP-seq data were generated to identify TCF7L2 target genes. Specifically, TCF7L2 protein expression was knocked down (KD) by siRNA transfection. After the KD efficiency had been validated by both qRT-PCR and western blot assays, RNA-seq was performed with control and KD samples to identify transcriptome-wide DEGs. ChIP-seq was also performed to identify TCF7L2 genome-wide DNA-binding sites. RNA-seq and ChIP-seq data were integrated as described previously [53] for the identification of TCF7L2 target genes. See the Supplementary Text for details.

TCF7L2 and BD risk genes

To annotate TCF7L2 protein function, the RNA-seq and ChIP-seq identified TCF7L2 target genes in hiPSC-derived astrocytes were subjected to pathway enrichment analysis. Those TCF7L2 target genes enriched BD as the top disease pathway. Expression levels of the BD risk genes quantitated by RNA-seq were plotted, and the TCF7L2-binding sites that mapped to or near the BD risk genes identified by ChIP-seq were visualized by using the Integrative Genomics Viewer [54]. See the Supplementary Text for details.

RESULTS

TCF7L2 gene transcription in human brain cells

To determine which CNS cell type(s) might express TCF7L2, we first consulted human brain snRNA-seq data generated by the Allen Institute [47]. Their data set included single-nucleus transcriptionomes of 49,495 nuclei across multiple human brain cortical areas. Based on marker gene expression, cells were “clustered” into seven non-neuronal and two major neuronal cell types (Fig. 1a). TCF7L2 was more highly expressed in astrocytes than in any of the other “clustered” cell types (Fig. 1b, c). Previously we had also analyzed scRNA-seq data [55] from two data sets generated from surgically removed human cerebral cortical tissue [48, 49] (Supplementary Fig. S1). In those studies, TCF7L2 was also expressed predominantly in astrocytes, e.g., 75.2% of astrocytes were TCF7L2 positive, a percentage nearly twice that of any other CNS cell type (Fig. 1d). The high levels of TCF7L2 expression in astrocytes were supported by...
scATAC-seq data generated from 70,631 human brain cells [50]. The human brain scATAC-seq data showed that TCF7L2 chromatin accessibility in astrocytes was higher than that for other clustered CNS cell types (Fig. 1e), compatible with highly active TCF7L2 transcription in astrocytes. Since TCF7L2 is most highly expressed in astrocytes, and since insulin signaling in astrocytes is known to co-regulate CNS glucose sensing and systemic glucose metabolism [56, 57], we next set out to characterize TCF7L2 function in hiPSC-derived astrocytes in an effort to understand its possible role in the comorbidity of BD and obesity.

The BMI-dependent BD risk SNP regulates brain-specific TCF7L2 transcript variant expression through glucocorticoid signaling

We began this series of experiments by attempting to understand the TCF7L2 gene structure and the SNP signal that had initially suggested a possible relationship of TCF7L2 to BD risk. The Ensembl [58] human genome assembly (GRCh38/p13) annotated 26 human TCF7L2 exons (numbered in Fig. 2a) which gave rise to 30 possible transcript variants (see Supplementary Tables S2 and S3). The most highly expressed TCF7L2 transcript across human tissues is a protein-coding transcript (ENST00000369397) consisting of 14 exons [30]. We designated that transcript as the TCF7L2 reference transcript (Fig. 2a).

Alternative exons that mapped to intronic regions of the reference transcript were named on the basis of intron number, followed by a letter of the alphabet (see Fig. 2a). The TCF7L2 rs12772424 SNP that was associated with the BD-BMI interaction is independent of the TCF7L2 rs7903146 T2D risk SNP, e.g., they are not in LD in any racial or ethnic groups ($r^2 < 0.035$, $D' < 0.282$). Unlike the T2D risk rs7903146 SNP, which is an eQTL for TCF7L2 RNA levels in multiple tissues [30], the rs12772424 SNP is not an eQTL for TCF7L2 in any tissue or organ [30]. However, we noticed that the variant allele for the rs12772424 SNP created a half-palindrome GRE (see Fig. 2a), a DNA motif that binds to the ligand-activated GR, which could then either enhance or repress gene transcription [59]. We also noted that eleven additional GREs were present in the “LD block” that includes rs12772424.
To determine whether the GR or other GR-related transcription factors (TFs) might bind to this “LD block”, we consulted the ENCODE [60] database that contains ChIP-seq data generated using A549 lung cancer cells after treatment with the GR agonist, DEX. Those ChIP-seq data showed that GR (encoded by NR3C1), FOXA1, POLR2A, and CTCF were all bound to this haplotype block (Fig. 2b). These observations raised the possibility that TCF7L2 transcription might be influenced by glucocorticoid signaling. As a first step, we measured RNA levels for all annotated TCF7L2 transcripts in A549 cells since ChIP-seq showed that GR and other TFs bound near the rs12772424 SNP in A549 cells after treatment with 100 nM DEX (Fig. 2b). The RNA level of FKBP5, a prototypic
GR-inducible gene, was also assayed as a positive control for response to DEX treatment. As expected, FKBP5 RNA expression increased significantly after DEX treatment (Supplementary Fig. 5a). However, the expression of three of the possible TCF7L2 transcript variants, including “T-2” and “T-3”, was significantly downregulated, whereas the expression of other TCF7L2 potential transcript variants was not changed significantly (Supplementary Fig. 5a) nor were they detectable by qRT-PCR (data not shown). Furthermore, repression of the “T-2” and “T-3” transcripts was DEX-dose dependent (Supplementary Fig. 5b). In an attempt to validate these observations for A549 cells by using CNS cell lines, we cultured human iPSC-derived astrocytes and pancreatic β cells (cell characterization shown in Supplementary Fig. 53), which showed that “T-3” was highly expressed in hiPSC-derived astrocytes, hepatocytes, and pancreatic β cells (cell characterization shown in Supplementary Fig. 53), which showed that “T-3” was highly expressed in hiPSC-derived astrocytes but not in two peripheral cell models (Fig. 3d). Western blot analysis further confirmed the decrease in TCF7L2 protein after “T-3” KD in hiPSC-derived astrocytes (Fig. 3h), an observation that was reproduced in two additional cell lines that express “T-3” (Supplementary Fig. 54). When the DEGs after T-3 KD were used to perform pathway enrichment analysis using Enrichr [52], the “top” enriched pathway in the DEGs after T-3 KD was the unfolded protein response (UPR) pathway (see Fig. 3g). This result also explains why, when RNA-seq is performed with poly(A) selected cDNA libraries which capture mainly protein-coding RNA, including the library used by GTEX [30] and the scRNA-seq data that we analyzed [48, 49], “T-3” could not be detected.

In an effort to study the possible function of “T-3”, we first quantified “T-3” expression in hiPSC-derived astrocytes, hepatocytes, and pancreatic β cells (cell characterization shown in Supplementary Fig. 53), which showed that “T-3” was highly expressed in hiPSC-derived astrocytes but not in two peripheral cell models (Fig. 3d). The presence of “T-3” in hiPSC-derived astrocytes was further confirmed by cDNA amplification using total RNA as a template (Fig. 3e). “T-3” was then KD in hiPSC-derived astrocytes by using antisense oligonucleotides (ASO) targeting exon-4d. The “T-3” KD efficiency was >80% in astrocytes transfected with pooled ASOs (Fig. 3f). That RNA sample, together with a non-targeting ASO control, was then used to perform RNA-seq. “T-3” KD in astrocytes resulted in a total of 1363 DEGs with fold change (FC) ≥ 2 (FDR < 0.05), which included TCF7L2 poly(A)+/mRNA (see Fig. 3g and Supplementary Table S6). Western blot analysis further confirmed the decrease in TCF7L2 protein after “T-3” KD in hiPSC-derived astrocytes (Fig. 3h), an observation that was reproduced in two additional cell lines that express “T-3” (Supplementary Fig. 54). When the DEGs after T-3 KD were used to perform pathway enrichment analysis using Enrichr [52], the “top” enriched pathway in the BioPlanet database [61] was “unfolded protein response” (Fig. 3i), an endoplasmic reticulum stress response pathway which is well-known to play a role in diabetes, metabolic syndrome and neurodegenerative disorders [62]. Diabetes and insulin signaling pathways were also identified by the pathway enrichment analysis (Fig. 3i). Those DEGs also identified “waist circumference adjusted for BMI” as the “top” associated phenotype in the GWAS Catalog [63] (Fig. 3j), a phenotype that is a clinical measure related to obesity and related disease risk [64]. In summary, we experimentally validated the existence of the TCF7L2 long non-coding transcript “T-3” in the human brain and, particularly, in hiPSC-derived astrocytes. The RNA-seq analysis after “T-3” KD indicated that this IncRNA might contribute to the regulation of the expression of genes related to TCF7L2 SNPs and glucocorticoid-regulated transcript variants. a TCF7L2 gene structure. TCF7L2 exons are numbered from 1 to 14 based on the reference RNA transcript (ID: ENST00000369397). Alternative exons that map to introns of the reference transcript were named using the intron number, followed by a letter of the alphabet. Positions of the rs79303146 SNP and the rs12772424 SNP are indicated and they map 122.2 kb distant from each other. The rs12772424 SNP variant allele (A) created a half-palindrome glucocorticoid response element (GRE). b The rs12772424 SNP loci. Positions of rs12772424 and its “linked” SNPs (r2 > 0.35, D’ > 0.79 in European population) are labeled. TCF7L2 exons are depicted as boxes with exon numbers labeled above. Putative GREs are indicated by gray boxes with boxes below the GREs. Darker colors represent stronger binding. c TCF7L2 transcript variants (T-1 to T-3) annotated by Ensembl are shown with their IDs shown in parentheses. d Immunofluorescent staining of astrocyte markers, GFAP and S100B, for hiPSC-derived astrocytes. Scale bars represent 50 μm. e Fold changes in RNA levels for TCF7L2 transcript variants in hiPSC-derived astrocytes after treatment with 100 nM DEX. Unique exon junctions (“J”) for specific transcript variants were quantified by qRT-PCR. GAPDH and FKBP5 expression was measured as internal and treatment controls, respectively. Data are mean ± s.d. (n = 3). ***p < 0.001 by Dunnett’s test. f DEX-dose-dependent induction of FKBP5 and repression of “T-2” and “T-3” in hiPSC-derived astrocytes. g Construction of reporter gene plasmid. DNA fragments containing the rs12772424 SNP and exon 4b-4d of cells. h Luciferase assays after cells were transfected with plasmids containing rs12772424 wild type (T) or variant (A) alleles and were treated with DEX or vehicle control. Data are presented as relative luciferase units (RLUs) after DEX-dose-dependent induction of FKBP5 and repression of “T-2” and “T-3” in hiPSC-derived astrocytes. i FACT that the rs12772424 SNP creates a GRE. j The rs12772424 through GR signaling which is highly expressed in the human brain but not in the other known to play a role in diabetes, metabolic syndrome and neurodegenerative disorders [62]. Diabetes and insulin signaling pathways were also identified by the pathway enrichment analysis (Fig. 3i). Those DEGs also identified “waist circumference adjusted for BMI” as the “top” associated phenotype in the GWAS Catalog [63] (Fig. 3j), a phenotype that is a clinical measure related to obesity and related disease risk [64]. In summary, we experimentally validated the existence of the TCF7L2 long non-coding transcript “T-3” in the human brain and, particularly, in hiPSC-derived astrocytes. The RNA-seq analysis after “T-3” KD indicated that this IncRNA might contribute to the regulation of the expression of genes related to TCF7L2 SNPs and glucocorticoid-regulated transcript variants. a TCF7L2 gene structure. TCF7L2 exons are numbered from 1 to 14 based on the reference RNA transcript (ID: ENST00000369397). Alternative exons that map to introns of the reference transcript were named using the intron number, followed by a letter of the alphabet. Positions of the rs79303146 SNP and the rs12772424 SNP are indicated and they map 122.2 kb distant from each other. The rs12772424 SNP variant allele (A) created a half-palindrome glucocorticoid response element (GRE). b The rs12772424 SNP loci. Positions of rs12772424 and its “linked” SNPs (r2 > 0.35, D’ > 0.79 in European population) are labeled. TCF7L2 exons are depicted as boxes with exon numbers labeled above. Putative GREs are indicated by gray boxes with boxes below the GREs. Darker colors represent stronger binding. c TCF7L2 transcript variants (T-1 to T-3) annotated by Ensembl are shown with their IDs shown in parentheses. d Immunofluorescent staining of astrocyte markers, GFAP and S100B, for hiPSC-derived astrocytes. Scale bars represent 50 μm. e Fold changes in RNA levels for TCF7L2 transcript variants in hiPSC-derived astrocytes after treatment with 100 nM DEX. Unique exon junctions (“J”) for specific transcript variants were quantified by qRT-PCR. GAPDH and FKBP5 expression was measured as internal and treatment controls, respectively. Data are mean ± s.d. (n = 3). ***p < 0.001 by Dunnett’s test. f DEX-dose-dependent induction of FKBP5 and repression of “T-2” and “T-3” in hiPSC-derived astrocytes. g Construction of reporter gene plasmid. DNA fragments containing the rs12772424 SNP and exon 4b-4d of cells. h Luciferase assays after cells were transfected with plasmids containing rs12772424 wild type (T) or variant (A) alleles and were treated with DEX or vehicle control. Data are presented as relative luciferase units (RLUs) after DEX-dose-dependent induction of FKBP5 and repression of “T-2” and “T-3”.
metabolic phenotypes, a link to the BD-BMI phenotype identified during our original GWAS in which rs12772424 was the “top hit” SNP.

Integration of TCF7L2 RNA-seq and ChIP-seq identified genes that play a role in CNS development

TCF7L2 mRNA was more highly expressed in astrocytes than in other CNS cell types (Fig. 1). However, the function of TCF7L2 as a TF in astrocytes remained unclear. Since TCF7L2 mRNA and protein levels were significantly decreased after “T-3” KD (Fig. 3g, h), we next performed TCF7L2 RNA-seq and ChIP-seq to identify TCF7L2 target genes in hiPSC-derived astrocytes. TCF7L2 mRNA was efficiently (>80%) KD by siRNA (Fig. 4a), which mainly targets cytoplasmic mRNA [65]. KD of TCF7L2 protein was confirmed by western blot assay (Fig. 4b). DEGs were identified by the comparison of RNA-seq data obtained from astrocytes transfected with non-targeting control and those transfected with TCF7L2 siRNAs (Fig. 4c). We also performed TCF7L2 ChIP-seq to identify the DNA-binding sites for this TF across the genome of hiPSC-derived astrocytes (Fig. 4d). A total of 1858 TCF7L2 ChIP peaks were identified, approximately one-third of which mapped to promoters and transcription start and termination sites (TSS and TTS) (Fig. 4e). As expected, DNA motif analysis identified the prototypic TCF7L2-binding motif as the “top hit”, supporting the specificity of the ChIP-seq results (Fig. 4f). The ChIP-seq peaks were then integrated with RNA-seq-identified DEGs [53] to identify genes directly targeted by TCF7L2 (Fig. 4g, Supplementary Table S7). Those 186 genes included TCF7L2 itself, which was previously known to be bound by TCF7L2 protein [66], the AXIN2 gene that is a prototypic Wnt target gene [67], and other genes that are known to be
involved in Wnt signaling (Fig. 4b). When the 186 TCF7L2 target genes in hiPSC-derived astrocytes were subjected to pathway enrichment analysis, as expected, the Wnt signaling pathway was identified as one of the “top” biological pathways (Fig. 4i). In addition, developmental pathways, including nervous system development and neurogenesis pathways, were enriched by the 186 TCF7L2 target genes (Fig. 4i), a result consistent with the known role of TCF7L2 in Wnt signaling and in cell development.
These results provided evidence which suggested that TCF7L2 may play a role in human CNS development, a process that has been linked to BD pathophysiology [68].

**TCF7L2 directly regulates BD risk genes**

To further investigate possible disease pathway(s) that might be related to the 186 TCF7L2 target genes in astrocytes, disease pathway enrichment was performed. BD was the most significantly enriched disease for these genes (FDR < 0.05) based on the Jensen disease database [69] (Fig. 5a). Eight of the enriched BD genes were NCAN, TENM4 (previously named ODZ4), FBLN1, ZMIZ1, NFIA, ZSWIM6, DOK5, and SLC45A4. Expression of all eight of these BD risk genes was significantly changed after TCF7L2 KD (Fig. 5b). Finally, ChIP-seq demonstrated that TCF7L2 bound directly to sequences in or near all of these genes, suggesting direct regulation of their expression (Fig. 5c). Integration of RNA-seq and ChIP-seq improved the accuracy of the identification of TCF7L2 target genes, e.g., TCF7L2 ChIP peaks mapped ~100 kb away from NCAN and SLC45A4 (Fig. 5c) but based on the RNA-seq data, no other genes that mapped nearer to those peaks were differentially expressed in hiPSC-derived astrocytes after TCF7L2 KD (Supplementary Table S7), suggesting possible direct regulation of NCAN and SLC45A4 expression. Seven of the TCF7L2-regulated BD risk genes shown in Fig. 5b, with the exception of SLC45A4, which encodes a sucrose transporter, are known to function in CNS cellular differentiation or neurodevelopment (Supplementary Table S8). Furthermore, seven of those eight genes (all except TENM4) contain genetic polymorphisms that are genome-wide significantly associated with either BMI or metabolic phenotypes (see Supplementary Table S8). These results suggested that TCF7L2 may directly regulate genes associated with BD risk and BMI or metabolic phenotypes in astrocytes, a possible molecular mechanism related to the role of TCF7L2 in the comorbidity of BD and obesity.
DISCUSSION

Metabolic comorbidities are highly prevalent in BD and are associated with more severe mood symptoms and worse treatment outcomes [16–19]. Disrupted insulin signaling in the brain and the hypothalamic–pituitary–adrenal axis has been proposed as one explanation for the comorbidity of mood disorders and obesity [70]. Recent large GWA studies of BD risk [6, 7] and obesity [71] have provided additional evidence for genetic correlations between BD and metabolic syndromes. Specifically, pathway enrichment of GWAS-identified BD risk genes often identified metabolic pathways such as insulin signaling and energy metabolism [6, 7]. Moreover, pathway analyses of BMI-related genes have strongly supported a role for the CNS in obesity susceptibility [71]. Obesity management by pharmacologic treatment in patients with psychiatric disorders is presently being tested as a novel therapeutic intervention [72]. In an effort to understand the possible contribution of genetics to the comorbidity of BD and obesity, we previously performed a GWAS for BD risk incorporating interaction with BMI, a study in which we identified a genome-wide significant SNP (rs12772424) associated with BMI-related BD that mapped to the TCF7L2 gene [20]. In the present study, we functionally characterized TCF7L2 in astrocytes, CNS cells that highly express TCF7L2 (Fig. 1), in an attempt to understand its possible role in the comorbidity of BD and obesity. Our results suggest that the TCF7L2 gene might play a role in both CNS development and in the regulation of metabolism in astrocytes, at least in part through the actions of different TCF7L2 variant transcripts.

TCF7L2 has been studied extensively as a TF in peripheral tissues with a role in glucose metabolism and T2D risk. The TCF7L2 protein has also been well characterized and includes a β-catenin-binding domain, suggesting a role in the canonical Wnt pathway (or Wnt/β-catenin pathway) [73]. Our studies have confirmed its role in Wnt signaling in hiPSC-derived astrocytes (Fig. 4), a function that might be related to BD risk (see Fig. 5). However, TCF7L2 transcription is complicated, e.g., with a total of 30 transcript variants listed in the Ensembl database. More than half of those transcript variants do not include exons 1–4 (Fig. 2a), which encode the β-catenin-binding domain [73], supporting Wnt-independent TCF7L2 functions that have been observed in the mouse brain [38]. The TCF7L2 rs12772424 SNP that we reported led us to a series of IncRNA-TCF7L2 transcripts that do not include exons 1–4, and specifically to “T-3”, which is highly expressed in the human brain and with an expression that is regulated by glucocorticoid signaling (Fig. 2). IncRNAs are known to affect the expression of a large number of genes by both cis- and trans-regulation [74] and to play key roles in various brain disorders [75]. Our functional characterization of “T-3” in hiPSC-derived astrocytes revealed a role in the regulation of the expression of genes involved in diabetes and insulin signaling pathways (Fig. 3), results which suggest that in the CNS TCF7L2, in addition to its role in the liver [32–34], pancreas [35, 36], and gut [37], might also contribute to metabolic regulation.

Abnormal cortisol levels in BD patients have been observed during DE/CRH testing [76] and the GR is expressed in astrocytes [77]. Astrocytes have recently been recognized as key players in neuropsychiatric disorders, including BD [78, 79], and they have been proposed as cellular targets for BD pharmacotherapy [80]. Recent studies have demonstrated that dysfunction of insulin signaling in hypothalamic astrocytes can influence systemic glucose levels in mice [56, 57]. Of course, these results with experimental animals will also have to be pursued in human subjects. Our identification of the “T-3” glucocorticoid-responsive IncRNA-TCF7L2 (Fig. 2), a non-coding transcript that regulates the expression of both TCF7L2 mRNA and of genes involved in insulin signaling pathways in hiPSC-derived astrocytes (Fig. 3) may help to explain, in part, the correlation of glucocorticoid signaling, insulin signaling, and BMI in BD.

Finally, we should point out the limitations of our studies, beginning with our use of cell line models. We used hiPSC-derived astrocytes to study TCF7L2 molecular function because TCF7L2 is more highly expressed in astrocytes than in other CNS cell types and, to our knowledge, its function in human CNS cell model(s) has not been reported previously. However, TCF7L2 is also expressed in neurons and oligodendrocyte progenitor cells (see Fig. 1), and it may regulate oligodendrocyte differentiation in mice [38, 39]. Whether TCF7L2 in other CNS cell types might contribute to BD and/or BMI is unknown and should be the subject of future studies. However, our data strongly suggest that TCF7L2 may play dual roles in the regulation of astrocytic insulin signaling and CNS development, perhaps through the effects of different RNA transcripts, a possibility that should also be pursued in the future. Obviously, molecular mechanisms based on cell line model systems are not able to provide a comprehensive view of physiological processes underlying clinical phenotypes. Therefore, genetically modified animal models and/or three-dimensional human brain organoids represent logical next steps in pursuit of the observations reported here. We should also point out that, although BMI was the best obesity-related phenotype that we had for all of our GWAS patients, we appreciate that it is not an ideal measure of obesity [21] and that other phenotypes such as waist circumference could provide complementary information on obesity-related phenotypes. In this context, it is of interest that “waist circumference adjusted for BMI” emerged as a top GWAS Catalog phenotype in our pathway enrichment analysis using DEGs after knockdown of the IncRNA-TCF7L2 transcript in astrocytes (see Fig. 3).

In summary, by setting out to define the molecular function of TCF7L2 in hiPSC-derived astrocytes, we have demonstrated that glucocorticoid regulation of the transcription of a TCF7L2 encoded non-coding transcript in a SNP-dependent fashion might influence astrocytic insulin signaling pathways, an effect which could also influence systemic glucose metabolism. We also obtained evidence that astrocytic TCF7L2 might contribute to BD pathophysiology by regulating the expression of BD risk genes. These results strongly support the possibility that the function of TCF7L2, and specifically the function of the IncRNA-TCF7L2 T-3 variant transcript in the CNS might represent an important link between BD, BMI, and metabolism.

DATA AVAILABILITY

The RNA-seq and ChIP-seq data sets generated during the current study are available in the Gene Expression Omnibus with accession ID: GSE17922.

REFERENCES

1. Frye MA. Bipolar disorder — a focus on depression. N Engl J Med. 2011;364:51–9.
2. Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. Arch Gen Psychiatry. 1997;54:313–21.
3. Oquendo MA, Currier D, Liu SM, Hasin DS, Grant BF, Blanco C. Increased risk for suicidal behavior in comorbid bipolar disorder and alcohol use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). J Clin Psychiatry. 2010;71:902–9.
4. Frye MA, Altschuler LL, McElroy SL, Suppes T, Keck PE, Denicoff K, et al. Gender differences in prevalence, risk, and clinical correlates of alcoholism comorbidity in bipolar disorder. Am J Psychiatry. 2003;160:883–9.
5. McElroy SL, Frye MA, Helleman G, Altschuler L, Leverich GS, Suppes T, et al. Prevalence and correlates of eating disorders in 875 patients with bipolar disorder. J Affect Disord. 2011;128:191–8.
6. Stahl EA, Breen G, Forstner AJ, McQuillin A, Ripke S, Trubetskoy V, et al. Genome-wide association study identifies 30 loci associated with bipolar disorder. Nat Genet. 2019;51:793–803.
7. Li HJ, Zhang C, Hui L, Zhou DS, Li Y, Zhang CY, et al. Novel risk loci associated with genetic risk for bipolar disorder among Han Chinese individuals: a genome-wide association study and meta-analysis. JAMA Psychiatry. 2021;78:320–30.
56. García-Cáceres C, Quarta C, Varella L, Gao Y, Gruber T, Legutko B, et al. Astrocytic insulin signaling couples brain glucose uptake with nutrient availability. Cell. 2016;166:867–80.

57. García-Cáceres C, Balland E, Prevot V, Luquet S, Woods SC, Koch M, et al. Role of astrocytes, microglia, and tanyocytes in brain control of systemic metabolism. Nat Neurosci. 2019;22:7–14.

58. Yates AD, Achuthan P, Akanni W, Allen J, Allen J, Alvarez-Jarreta J, et al. Ensembl 2020. Nucleic Acids Res. 2020;48:D682–D8.

59. Weikum ER, Knuesel MT, Ortlund EA, Yamamoto KR. Glucocorticoid receptor control of transcription: precision and plasticity via allosteroy. Nat Rev Mol Cell Biol. 2017;18:159–74.

60. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res. 2018;46:D794–801.

61. Huang R, Grishagin I, Wang Y, Zhao T, Greene J, Obenauer J, et al. The NCATS BioPlanet - an integrated platform for exploring the universe of cellular signaling pathways for toxicology, systems biology, and chemical genomics. Front Pharmac. 2019;10:445.

62. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol. 2012;197:857–67.

63. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. 2019;47:D1005–D1012.

64. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol. 2020;16:177–89.

65. Hannon GJ. RNA interference. Nature. 2002;418:244–51.

66. Davis CA, Hitz BC, Sloan CA, Chan ET, Davidson JM, Gabdank I, et al. The Encyclopedia of DNA elements (ENCODE): data portal update. Nucleic Acids Res. 2018;46:D794–801.

67. Yan D, Wiesmann M, Rohan M, Chan V, Jefferson AB, Guo L, et al. Elevated expression of axin2 and hmxd mRNA provides evidence that Wnt/beta-catenin signaling is activated in human colon tumors. Proc Natl Acad Sci USA. 2001;98:4973–8.

68. Madison JM, Zhou F, Nigam A, Hussain A, Barker DD, Nehme R, et al. Characterization of bipolar disorder patient-specific induced pluripotent stem cells from a family reveals neurodevelopmental and mRNA expression abnormalities. Mol Psychiatry. 2015;20:703–17.

69. Pletscher-Frankild S, Palleja A, Tsafou K, Binder JX, Jensen LJ. DISEASES: text mining and data integration of disease-gene associations. Methods. 2015;74:3–9.

70. Nguyen TTL, Chan LC, Borreginne K, Kale RP, Hu C, Tye SJ. A review of brain insulin signaling in mood disorders: from biomarker to clinical target. Neurosci Biobehav Rev. 2018;92:7–15.

71. Locke AE, Khalahi B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518:197–206.

72. Madison JM, Zhou F, Nigam A, Hussain A, Barker DD, Nehme R, et al. Characterization of bipolar disorder patient-specific induced pluripotent stem cells from a family reveals neurodevelopmental and mRNA expression abnormalities. Mol Psychiatry. 2015;20:703–17.

73. Whischer CA, Price HC, Phiri P, Rathod S, Barnard-Kelly K, Reidy C, et al. LIAglutide and the management of overweight and obesity in people with schizophrenia, schizoaffective disorder and first-episode psychotic: protocol for a pilot trial. Trials. 2019;20:633.

74. Poy F, Lepourcelet M, Shivdasani RA, Eck MJ. Structure of a human Tcf4-beta-catenin complex. Nat Struct Biol. 2001;8:1053–7.

75. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021;22:96–118.

76. Yang S, Lim KH, Kim SH, Joo JY. Molecular landscape of long noncoding RNAs in brain disorders. Mol Psychiatry. 2021;26:D1005–D8.

77. Vieta E, Martinez-De-Osaba MJ, Colom F, Martinez-Aran A, Benabarre A, Gasto C. Enhanced corticotropin response to corticotropin-releasing hormone as a predictor of mania in euthymic bipolar patients. Psychol Med. 1999;29:971–8.

78. Bohn MC, Howard E, Vilekind U, Krozowski Z. Glia cells express both mineralocorticoid and glucocorticoid receptors. J Steroid Biochem Mol Biol. 1991;40:105–11.