Genetic Variants in MicroRNAs and Their Binding Sites Are Associated with the Risk of Parkinson Disease

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ABSTRACT: MicroRNAs (miRNAs) are small noncoding RNAs that serve as key regulators of gene expression. They have been shown to be involved in a wide range of biological processes including neurodegenerative diseases. Genetic variants in miRNAs or miRNA-binding sites on their target genes could affect miRNA function and contribute to disease risk. Here, we investigated the association of miRNA-related genetic variants with Parkinson disease (PD) using data from the largest GWAS on PD. Of 243 miRNA variants, we identified rs897984:T>C in miR-4519 (P value = 1.3×10⁻⁵ and OR = 0.93) and rs11651671:A>G in miR-548at-5p (P value = 1.1×10⁻⁶ and OR = 1.09) to be associated with PD. We showed that the variant’s mutant alleles change the secondary structure and decrease expression level of their related miRNAs. Subsequently, we highlighted target genes that might mediate the effects of miR-4519 and miR-548at-5p on PD. Among them, we experimentally showed that NSF is a direct target of miR-4519. Furthermore, among 48,844 miRNA-binding site variants, we found 32 variants (within 13 genes) that are associated with PD. Four of the host genes, CTSSB, STX1B, IGF5P9B, and HSD3B7, had not previously been reported to be associated with PD. We provide evidence supporting the potential impact of the identified miRNA-binding site variants on miRNA-mediated regulation of their host genes.


KEY WORDS: Parkinson disease; GWAS; miRNA variant; miRNA-binding site variant

Introduction

Neurodegenerative diseases collectively represent one of the major worldwide causes of morbidity and healthcare costs to society [Cowan and Kandel, 2001]. Parkinson disease (PD) is the second most common neurodegenerative disorder [de Lau and Breteler, 2006], and its prevalence and burden at the population level are projected to grow dramatically as the size of elderly population increases [Dorsey et al., 2007]. Clinically, PD is characterized by a combination of motor symptoms, known as parkinsonism, and a range of nonmotor symptoms, such as cognitive decline and autonomic dysfunction, that contribute to a devastating loss of quality of life [Lansbury and Lashuel, 2006; Lohle et al., 2009]. PD is thought to be a complex disease, and genetic factors have a substantial impact on the phenotypic variation of the disease [Shulman et al., 2011]. Over the past few decades, enormous efforts have been done to discover genetic factors that play a role in the development of PD. In recent years, the large-scale genome-wide association studies (GWAS) have enabled the discovery of hundreds of genetic variants that are associated with PD risk [Lill et al., 2012; Nalls et al., 2014]. Nevertheless, most of the identified variants are mapped to noncoding regions of the genome, and their causal mechanisms remain to be investigated.

MicroRNAs (miRNAs) are a class of small noncoding RNAs, that post-transcriptionally regulate gene expression [Ambros, 2004; Bartel, 2009]. miRNAs have been shown to be involved in a wide range of biological processes and human diseases including neurodegenerative disorders [Issler and Chen, 2015; Muller et al., 2015; Tan et al., 2015]. In addition, a number of dysregulated miRNAs have been reported to be associated with PD in patients and animal models [Kim et al., 2007; Harraz et al., 2011; Khoo et al., 2012]. The regulatory functions of miRNAs are accomplished through binding of the nucleotides 2–8 from their 5’end (the seed region) to the complementary sequences at the target miRNAs, resulting in repression of translation or a decreased stability of target mRNAs [Ambros, 2004; Bartel, 2009]. Genetic variants that fall within miRNA-related sequences may affect miRNA function and due to aberrant target genes expression, the variants could modify susceptibility to disease [Ryan et al., 2010]. Recently, we and others have been able to show a number of polymorphisms in miRNAs or their target genes that may contribute to phenotypic variations [Hughes et al., 2011; Gong et al., 2012; Richardson et al., 2013; Ghanbari et al., 2014; Ghanbari et al., 2015]. However, no systematic investigation of the impact of such variants on the risk of PD has been published to date.

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

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In this study, we examined the association of variants in miRNAs as well as miRNA-binding sites with PD using data from the largest GWAS. We subsequently integrated our GWAS findings with computational and biological information (such as miRNA and gene expression profiles) and performed experimental studies to provide evidence for functionality of the identified variants in PD.

Materials and Methods

Identification of Genetic Variants Located in miRNAs and miRNA-Binding Sites

We retrieved all genetic variants that are localized in miRNAs or miRNA-binding sites on their target genes by reviewing the literature and using two online databases: miRNASNP (v2.0) [Gong et al., 2015] and PolyMiRTS (v3.0) [Bao et al., 2007]. We retained a total of 2,420 variants in all human precursor (60–80 nt) and mature (20–24 nt) miRNA sequences. We excluded variants with minor allele frequency (MAF) < 0.01. Of the remaining variants, we included 243 single-nucleotide polymorphisms (SNPs) in 214 miRNAs that were present in the recent GWAS of PD [Nalls et al., 2014]. Furthermore, we retained around 401,000 miRNA-binding site variants that were predicted to affect the match to the seed region of miRNAs. Of these, 48,845 SNPs with MAF > 0.01 and present in the GWAS of PD were included [Nalls et al., 2014]. A flowchart of our approach to retrieve the variants located in miRNAs and miRNA-binding sites is shown in Figure 1.

GWAS on PD

We examined the association of retrieved variants in miRNAs and miRNA-binding sites with PD using summary statistics data from the largest GWAS on PD across 13,708 PD cases (39% female) and 95,282 controls (46% female) [Nalls et al., 2014]. The GWAS data were imputed to 1000 Genomes project reference panel, providing data for 7,893,274 variants. All participating studies in the PD-GWAS had provided informed consent for participation in genetics studies and were approved by their local ethical committees. More details about the consortium and participants are described elsewhere [Nalls et al., 2014]. We used the Bonferroni correction, to adjust \( P \) value for the number of tests, and significant threshold was set at \( 2.06 \times 10^{-4} \) (0.05/243) for variants in miRNAs and \( 1.02 \times 10^{-5} \) (0.05/48,844) for variants in miRNA-binding sites.

Analyzing the Variant Effect on miRNA Structure and Expression

For miRNA variants that were associated with PD, we used the Vienna RNAfold algorithm (ViennaRNA package 2.0) to predict the variant effect on the hairpin structure of miRNA [Lorenz et al., 2011]. Difference in minimum free energy (MFE) of the thermodynamic ensemble of precursors miRNA (pre-miRNA) sequence containing the mutant versus the wild-type allele may indicate an altered miRNA processing. Furthermore, to experimentally examine the variant’s effect on the expression level of mature miRNA, we cloned the pre-miRNA sequences containing either the wild-type or mutant allele behind the gene encoding green fluorescent protein (GFP) in the expression plasmid MSCV-BC, resulting in GFP—miRNA fusion transcripts [Meenhuis et al., 2011]. The inserts of all constructs were validated by Sanger sequencing. HEK293 cell transfection, total RNA isolation, and quantitative PCRs were performed as previously described [Meenhuis et al., 2011]. The primers are shown in Supp. Table S1. The experiment was performed in triplicate.

Quantitative PCR of miRNAs

We examined whether miRNAs hosting the variants associated with PD are expressed in the human brain. To this end, brain tissue was obtained from the Netherlands Brain Bank (Amsterdam, The Netherlands). All samples were free of neurological disease. For isolation of total RNA, five cryopreserved sections of 40 \( \mu \)m were homogenized in 250 \( \mu \)l Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was isolated from six brain samples (three white matter and three gray matter). The concentration and purity of RNA samples were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). The expression levels of miRNAs were determined with TaqMan MicroRNA Assays according to manufacturer’s protocols (Applied Biosystems, Foster City, CA). RNU6B was used as an endogenous control. The experiment was performed in triplicate.

Association of miRNA Target Genes with PD

For miRNAs that were associated with PD, we further examined which of the target genes may mediate the effect of miRNAs on PD. To do this, we extracted all predicted target genes of the identified miRNAs using two online databases, TargetScan v7.0 (http://www.targetscan.org) [Friedman et al., 2009] and miRDB (http://www.mirdb.org/miRDB) [Wong and Wang, 2015]. The miRNA target genes that are listed in both databases were used for our analyses. Next, we used the PD-GWAS data in a candidate gene approach to identify those target genes that are likely to be involved in developing PD. We retrieved the summary statistics for the association of all genetic variants in the target genes with PD. The significance threshold for this analysis was set using the Bonferroni correction based on the number of studied variants. Additionally, we performed ingenuity pathway analysis (IPA) to explore the pathways in which target genes of the identified miRNAs may play a role (http://www.ingenuity.com/products/ipa/). A list of all putative target genes of each miRNA was uploaded and a core IPA analysis was performed using the default settings. We mapped the miRNA target genes to biological functions or canonical pathways to determine whether they are enriched in neurological networks. The \( P \) values are calculated using the right-tailed Fisher’s exact test and a \( P \) value less than 0.05 indicates a statistically significant, nonrandom association.

Luciferase Reporter Assay

The pre-miRNAs were amplified and cloned in pMSCV-BC as previously described [Meenhuis et al., 2011]. The 3’UTR sequences of target gene (either wild-type or mutated), containing the putative binding site of the miRNA, were cloned downstream of the luciferase gene in the pGL3 vectors as previously described [Meenhuis et al., 2011]. The primers are shown in Supp. Table S1 and Supp. Table S2. The inserts of all constructs were validated by Sanger sequencing. COS cells were plated into 96-well plates and cotransfected with pMSCV-miRNA, pGL3 containing the different 3’UTRs, and a plasmid expressing the Renilla transfection control. Luciferase activity was determined with the Dual-Glo Luciferase Assay System according to manufacturer’s protocol (Promega, Madison, WI). Renilla
Figure 1. Identification of miRNA-related variants associated with Parkinson disease. This flow chart describes our selection process to retrieve variants located in miRNAs and miRNA-binding sites that are associated with PD. GWAS, genome-wide association study; PD, Parkinson disease; MAF, minor allele frequency; LD, linkage disequilibrium.

Luciferase activity was used for normalization. All experiments were performed five times.

Expression Quantitative Trait Loci

We scanned cis-expression quantitative trait loci (eQTL) data to examine the correlation between miRNA-binding site variants and the related transcript expression levels. We used two online Web browsers: Genenetwork (http://genenetwork.nl/bloodeqtlbrowser/) and GTEx V4 (http://www.broadinstitute.org/gtex/). The GTEx platform provides information on eQTL in different tissues including brain. In addition, we used the eQTL data in whole blood from the gene network because of a very large sample size (n = 5,311). The designs of these studies have been described in detail elsewhere [Grundberg et al., 2012; Westra et al., 2013].

Expression of the Identified Target Genes and Related miRNAs in Relevant Tissues

To search for expression of the identified target genes and their regulatory miRNAs in relevant tissues, we employed several Web tools. The Illumina’s Human Body Map 2.0 data (http://www.ensembl.info/blog/2011/05/24/human-bodymap-2-0-data-from-illumina/) were used to examine the expression of genes hosting miRNA-binding site variants across different tissues. This database provides RNASeq data of 16 human tissue types, including brain. To scan the expression of related miRNAs in the brain, we used the Human MiRNA Expression Database (HMED) [Gong et al., 2014], mimiRNA [Ritchie et al., 2010], and PhenomIR [Ruepp et al., 2010] databases. We further searched the literature via PubMed using the search terms of miRNA name and “expression” for those not implicated in the listed databases.

Analyzing Functional Characteristics of the Identified miRNA-Binding Site Variants

We evaluated LD blocks of the identified miRNA-binding site variants to examine whether there are other SNPs in high LD in the related loci that may drive the observed GWAS associations. To this end, the list of PD-associated variants in miRNA-binding sites were submitted to the SNAP Web tool (http://www.broadinstitute.org/mpg/snap/id) using R^2 threshold > 0.8, limit distance 500 kb, and population panel CEU to retrieve their proxy SNPs in the 1000 G. We then utilized the HaploReg Web tool v3 (http://www.broadinstitute.org/
mammals/haploreg/haploreg_v3.php) to predict the effect of SNPs on protein structure, gene regulation, and splicing. Other information, including miRNA sequences, miRNA host genes, and miRNA conservation in different species was obtained from TargetScan v7.0 and miRBase (release 20) [Kozomara and Griffiths-Jones, 2014] databases.

Results

Two miRNA Variants Were Associated with PD

We studied the association of 243 SNPs (with MAF > 0.01) located in 214 miRNAs with PD. Of these, rs11651671 (Chr17:42494785,A>G) in miR-548at-5p (\(P\) value = \(1.06 \times 10^{-6}\) and OR = 1.09) and rs897984 (Chr16:30875322, T>C) in miR-5419 (\(P\) value = \(1.34 \times 10^{-5}\) and OR = 0.93) were significantly associated with PD. Supp. Table S3 shows miRNA variants that are associated with PD with a \(P\) value < 0.05. The forest association plots showing meta-analysis of the association of the two identified miRNA variants with PD are shown in Supp. Figure S1.

The Effect of Variants on MiR-4519 and MiR-548at-5p Structure and Expression

We generated the hairpin structures of miR-4519 and miR-548at-5p containing the wild-type or mutant alleles using the Vienna RNAfold algorithm [Lorenz et al., 2011]. We noted a +6.1 kcal/mol difference in the MFE of the predicted thermodynamic ensemble of the mutant versus the wild-type miR-548at-5p structure (Supp. Fig. S2), which may affect the processing of pre-miRNA. The predicted change in MFE of the thermodynamic ensemble of the hairpin structure of miR-4519 containing the mutant versus the wild type was -0.3 kcal/mol (Supp. Fig. S3). We then examined the expression levels of these miRNAs from two instances: the wild-type pre-miRNA and the mutant pre-miRNA. We cloned the pre-miRNA sequences (containing either wild-type allele or mutant allele) behind the GFP in the expression plasmid. Transient transfection of the miRNAs in HEK293 cells showed significantly reduced levels of the mature miRNAs from the mutant constructs relative to GFP compared with the wild-type constructs, where the rs897984 mutant allele reduced the expression level of miR-4519 by 90% (\(P\) value = 0.003) and the rs11651671 mutant allele decreased the expression level of miR-548at-5p by 30% (\(P\) value = 0.049) (Fig. 2).

Association of MiR-4519 and MiR-548at-5p Target Genes with PD

miRNAs act through regulation of their target gene expression. We thus assessed whether 342 putative target genes of miR-4519 and 676 putative target genes of miR-548at-5p are implicated in neurological pathways using IPA. This analysis indicated that several target genes of miR-4519 are directly or indirectly linked with Nervous System Development and Function Networks, Supp. Table S4. We then examined the association of genetic variants in all putative target genes of miR-4519 and miR-548at-5p with PD using GWAS data. We studied 76,457 SNPs in 342 target genes of miR-4519, with the significant threshold of \(6.5 \times 10^{-7}\), and found four target genes, NSF, TMEM163, CCNT2, and SHG12, to be associated with PD (Table 1). For miR-548at-5p, we assessed 153,018 SNPs in 676 target genes, with the significance threshold of \(3.3 \times 10^{-7}\), and identified GCH1, MMRN1, CCNT2, and DCUN1D1 to be associated with PD (Table 1). Using the Human Body Map 2.0 data, we showed that the highlighted target genes of miR-4519 and miR-548at-5p are expressed in the brain (Supp. Table S5). Next, we asked whether miR-4519 and miR-548at-5p are expressed in the brain. Our data showed that miR-4519 is expressed at detectable levels in both white and gray matter of the human brain (average Ct value gray matter: 32.9 and average Ct value white matter: 32.1) (Supp. Table S6). However, we could not detect miR-548at-5p in these tissues by qPCR. Subsequently, we examined whether miR-4519 control the expression level of its top identified target gene, NSF, in vitro. We generated expression vector containing the pre-miR-4519 sequence and cotransfected the construct with luciferase reporters containing the wild-type and mutant 3’ UTR of NSF. We found that over-expression of miR-4519 significantly decreases the luciferase activity of wild-type NSF reporter, compared with the mutated NSF reporter, \(P\) value = 0.0002 (Fig. 3). These data indicate that NSF is a direct target of miR-4519.
Multiple miRNA-Binding Site Variants Were Associated with PD

We examined the associations of 48,844 miRNA-binding site variants with PD (Fig. 4). Of these, 32 SNPs located in the 3’UTR of 13 genes were significantly associated with PD (P value <1.0 × 10^{-6}) (Table 2). These SNPs are predicted to affect miRNA-mediated regulation of their host genes by creating, changing or modifying a number of miRNA-binding sites that are depicted in Supp. Tables S7 and S8. Out of 13 genes hosting the 32 SNPs, the association of nine genes with PD had already been reported by GWAS [Nalls et al., 2014]. Four others that were not previously reported for PD include HSD3B7 (P value = 5.2 × 10^{-8}), IGSF9B (P value = 2.6 × 10^{-7}), CSTB (P value = 4.8 × 10^{-7}), and STXB1 (P value = 1.2 × 10^{-7}).

Supporting Evidence for the Identified miRNA-Binding Site Variants

We searched for cis-eQTL of 13 genes hosting the 32 identified miRNA-binding site variants and found six SNPs that are correlated with expression levels of their host genes. This includes rs10420958, which was associated with SPP1L2B expression in the brain, and five SNPs (including rs708723 and rs2645425) that were associated with expression levels of CTTSB and RAB7L1 in blood. Using the HaploReg Web tool (v3), we searched for potential functionality of the 32 SNPs and their proxies in high LD on gene regulation. This analysis showed that some of the miRNA-binding site SNPs have no proxy or only weak proxies in the related loci, such as rs8327, rs356165, rs708723, and rs1128402 (Table 3). Furthermore, through scanning the expression of 13 host genes (using the Human Body Map data), we showed that SNCA, CTTSB, MAPT, and STXB1 are abundantly expressed in the brain (Supp. Table S9). We also found evidence for expression of several of the regulatory miRNAs in the brain, in particular miR-342-3p, miR-29a-5p, miR-9-5p, using the miRNA expression databases (Supp. Table S7). A summary of the evidence that we found suggesting the potential functionality of the
identified miRNA-binding site SNPs in their related loci is depicted in Table 3.

**Discussion**

In this study, we investigated the association of variants that are located in miRNAs and miRNA-binding sites with PD using population-level data. We found two common miRNA variants, rs897984 in miR-4519 and rs11651671 in miR-548at-5p, that are associated with PD. We showed that the variant’s mutant alleles have the potential to affect the hairpin secondary structures of pre-miRNAs and decrease the expression levels of mature miR-4519 and miR-548at-5p. We subsequently suggested target genes that might mediate the effects of miR-4519 and miR-548at-5p on PD. Among them, we experimentally showed that NSF is a direct target of miR-4519. Furthermore, we identified 32 miRNA-binding site variants (hosting by 13 genes) that are associated with PD. Four of the host genes, CTSB, STX1B, IGSF9B, and HSD3B7, had not previously been reported to be associated with PD. Finally, we provide evidence supporting some of the identified miRNA binding site variants to affect miRNA-mediated regulation of their host genes.

An increasing number of studies have shown the critical role of miRNAs in neurodegenerative disorders including PD [Hebert and De Strooper, 2007; Martins et al., 2011; Serafin et al., 2015]. Most of these studies have focused mainly on differentially expressed miRNAs and genes detected by expression arrays in a small sample size. In addition, some studies have linked a number of miRNAs with neurodegenerative disorders using the candidate gene approach. For example, Saba et al. (2014) have recently reported a catalog of SNPs overlapping miRNA-binding sites in a subset of genes that are implicated in neurological diseases. Here, we systematically investigated the association of all miRNA-related genetic variants with PD using population level data in a large scale.

The GWAS data show that carriers of the rs897984 mutant allele in miR-4519 have a decreased risk of PD. Our functional experiments then showed that the mutant allele dramatically decreases the expression level of mature miR-4519. SNP rs897984 occurs 1 nt after the 5’ end of pre-miR-4519 and overlaps the site of Drosha cleavage. The maturation of miRNAs is a complex and highly regulated process, which is characterized by two-step sequential processing by RNase III enzymes, Drosha and Dicer [Denli et al., 2004; Winter et al., 2009]. It has been shown that polymorphisms residing within (±1) nt of the Drosha or Dicer cleavage sites may affect miRNA biogenesis [Sun et al., 2009; Obsteter et al., 2015]. Therefore, the observed effect of rs897984 on the miR-4519 level can be explained with the SNP impact on the processing of miR-4519 by Drosha enzyme. We showed that miR-4519 is expressed in both gray and white matter of the human brain (Supp. Table S5). Interestingly, data from the GTEx database (http://www.gtexportal.org/home/gene/mir4519) showed that miR-4519 is expressed in higher levels in substantia nigra, the primary area of the brain that is affected by PD [Croisier et al., 2005]. In agreement with our findings, two recent studies have also demonstrated this miRNA to be expressed in the human brain tissues [Hong et al., 2015; Stamova et al., 2015].
We suggested that the effect of miR-4519 on PD might be mediated by four target genes (NSF, TMEM163, CCNT2, and SH3GL2) that were found to be potentially associated with PD. Among them, NSF showed the most significant association with PD (in the GWAS data) and is the most abundantly expressed gene in the brain (in the Human Body Map data). We further experimentally showed that NSF is a direct target of miR-4519. NSF is known as a crucial factor in intracellular membrane fusion events, such as the fusion of synaptic vesicles with the presynaptic membrane during neurotransmission [Haas, 1998]. This gene has also been shown to be associated with different neurodegenerative disorders including PD [Lenzcz et al., 2007; Liu et al., 2011]. Furthermore, previous studies have shown that expression of NSF is decreased in PD substantia nigra [Simunovic et al., 2010; Elstner et al., 2011; Capurro et al., 2014]. These data indicate that the rs897984 mutant allele influences the risk of PD through altering the expression of these target genes.

We also identified 32 miRNA-binding site SNPs that are associated with PD. Among 13 genes hosting the variants, SNCA (alpha-synuclein) plays an important role for normal brain function and is a major risk factor for PD [Chandra et al., 2005]. Genetic variants in SNCA have been shown to be associated with the common sporadic form of PD [Mueller et al., 2005; Mizuta et al., 2006; Tan and Skipper, 2007]. However, some of the associated variants are mapped downstream of the SNCA gene and a direct functional effect is thus unlikely. SNP rs356165 is located in the 3′UTR of SNCA and is one of the top associated SNPs with PD in the related locus. We found that the rs356165 mutant allele is predicted to disrupt a binding site of miR-6508, presumably resulting in an elevated level of SNCA expression. An allele-specific regulation of SNCA by miR-6508 might serve as a functional explanation behind the association of rs356165 with PD. Future studies are needed to determine the effect of rs356165 on SNCA expression and the function of miR-6508 in PD patients. Conversely, our results showed that some of the identified miRNA-binding site SNPs improve the original recognition sites or create new binding sites for miRNAs. For example, rs1128402 is predicted to create a binding site for miR-29a-5p in the 3′UTR of SPPL2B. This variant is one of the top SNPs associated with PD in the related locus and has no non-synonymous proxy. In addition, several independent studies have shown miR-29a-5p to be involved in PD and other neurodegenerative diseases [Margs et al., 2011; Bellingham et al., 2012; Chandrasekaran and Bonchev, 2013; Villa et al., 2013]. Downregulation of SPPL2B by miR-29a-5p may be a functional reason for the identified association of rs1128402 with PD.
We suggested four new genes, *CTSB*, *STX1B*, *IGSF9B*, and *HSD3B7*, that are potentially associated with PD. Of these, *CTSB* (Cathepsin B1) encodes a protein that is known as amyloid precursor protein (APP) secretase and is involved in the proteolytic processing of APP. Incomplete proteolytic processing of APP has been suggested to be a causative factor in Alzheimer disease [Sundelof et al., 2010; Schechter and Ziv, 2011]. We found three miRNA-binding site SNPs in the 3’UTR of *CTSB* that are associated with PD. Of these, rs2645425 is predicted to create a potential binding site for miR-342-3p. Using blood eQTL analysis, we showed that rs2645425 mutant allele carriers have lower expression levels of *CTSB*. The expression data further demonstrated that the *CTSB* gene is abundantly expressed in the brain. Interestingly, miR-342-3p has also been shown to be upregulated in the brain of patients with prion disease [Montag et al., 2009; Bellingham et al., 2012] and has been suggested as a biomarker for Alzheimer’s disease [Tan et al., 2014]. The observed association of rs2645425 with PD thereby might be through increasing the miR-342-3p-dependent regulation of *CTSB*. Among the other three genes, *STX1B* (syntaxin-1B) has been found to be directly implicated in the process of calcium-dependent synaptic transmission in the rat brain and has been suggested to play an important role in the excitatory pathway of synaptic transmission [Smirnova et al., 1993]. *IGSF9B* is known as Immunoglobulin Superfamily Member 9B and has reported as a novel, brain-specific, homophilic adhesion molecule that is strongly expressed in GABAergic interneurons [Woo et al., 2013]. Finally, *HSD3B7* has been shown to be associated with congenital bile acid synthesis defect and liver diseases [Fischler et al., 2007]. This gene has also been linked with the etiology of Alzheimer disease through deactivation pathway of LXR ligands in the brain [Ogundare et al., 2010]. Collectively, we identified two miRNA variants and multiple miRNA-binding site variants that are associated with PD and could potentially affect miRNA-mediated regulation of several genes associated with risk of PD. These results may contribute to increase our understanding of the role of miRNAs in the etiology of PD. Our findings may also be of clinical importance as they suggest a number of miRNAs that modify gene expression profiles and affect PD risk. Experimental assays of the identified variants and miRNA profiling in PD patients will be the next step toward determining the functionality of the variants, related target genes, and miRNAs in PD.

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