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Analysis of the *Micro-RNA-133* and *PITX3* Genes in Parkinson's Disease†

Lorena de Mena,[1] Eliecer Coto,[1,2] Lucía F. Cardo,[1] Marta Díaz,[1] Marta Blázquez,[3] René Ribacoba,[3] Carlos Salvado,[3] Pau Pastor,[4] LLuis Samaranch,[4] Germán Moris,[3] Manuel Menéndez,[3] Ana I. Corao,[1] and Victoria Alvarez[1]*

[1]Genética Molecular, Hospital Universitario Central de Asturias, Oviedo, Spain

[2]Departamento de Medicina, Universidad de Oviedo, Oviedo, Spain

[3]Neurología, Hospitales Universitario Central Asturias, San Agustín-Avilés, and Alvarez Buylla-Mieres, Asturias, Spain

[4]Department of Neurology, Division of Neurosciences, Neurogenetics Laboratory, Center for Applied Medical Research, Clinica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain

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MicroRNAs are small RNA sequences that negatively regulate gene expression by binding to the 3′ untranslated regions of mRNAs. MiR-133b has been implicated in Parkinson's disease (PD) by a mechanism that involves the regulation of the transcription factor PITX3. The variation in these genes could contribute to the risk of developing PD. We searched for DNA variants in miR-133 and *PITX3* genes in PD patients and healthy controls from Spain. We found common DNA variants in the three miR-133 genes. Genotyping of a first set of patients (n = 777) and controls (n = 650) showed a higher frequency of homozygous for a miR-133b variant (−90 del A) in PD-patients (6/575; 1%) than in healthy controls (0/650) ($P = 0.03$). However, this association was not confirmed in a second set of patients (1/250; 0.4%) and controls (2/210; 1%). No common *PITX3* variants were associated with PD, although a rare missense change (G32S) was found in only one patient and none of the controls. In conclusion, we report the variation in genes of a pathway that has been involved in dopaminergic neuron differentiation and survival. Our work suggests that miR-133 and *PITX3* gene variants did not contribute to the risk for PD.

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INTRODUCTION

Micro RNAs (miRNAs) are 20–23 nucleotide RNAs that bind to the 3′ untranslated regions (3′ UTRs) of mRNAs, and regulate gene

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*Correspondence to:
Victoria Alvarez, Genética Molecular, Hospital Central Asturias-Maternidad, 33006 Oviedo, Spain. E-mail: victoria.alvarez@sespa.princast.es

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processed to the single-strand mature miRNA. Mature miRNAs are incorporated into the RNA induced silencing complex that regulates mRNA expression [Bartel, 2004].

MiRNAs can play a role in dopaminergic neurons (DN) differentiation and survival [Kosik, 2006; Hébert and De Strooper, 2007]. A quantitative analysis of 224 miRNAs identified a loss of miR-133b expression in PD subjects. This miRNA was specifically expressed in the midbrain of normal mice and markedly reduced in mice dopamine-deficiency models, and regulates the maturation and function of DNs within a negative feedback circuit that includes the transcription factor Pitx3 [Kim et al., 2007]. Recent reports have provided evidence for the association between *PITX3* polymorphisms and PD [Bergman et al., 2008; Fuchs et al., 2009; Haubenberger et al., 2009; Le et al., 2009].

DNA variants at miRNA genes and at the 3′ UTR regions where miRNAs bind have been associated with the risk for diseases [Duan et al., 2007; Li et al., 2009]. A DNA polymorphism in the 3′ UTR of FGF20 that disrupted a binding site for miR-433 and increased the translation of FGF20 was associated with PD [Wang et al., 2008]. However, this association was not detected by other authors [Clarimon et al., 2005; Wider et al., 2009].

We hypothesized that DNA variants in the miRNA-133 and *PITX3* genes could contribute to the risk for PD. We searched for DNA variants in these miRNA genes in PD-patients, and compared the genotype frequencies between patients and healthy controls.

METHODS

Patients and Controls

The study included a total of 1,027 Parkinson's patients (mean age at diagnosis 58 ± 13 years; 52% male) recruited in the period 2002–2008 by Neurologists from the Movement Disorder Units of four reference Hospitals (Central de Asturias, Mieres, Avilés, and Clínica Universitaria Pamplona) from two regions in Spain: Asturias and Navarra. The control group consisted of 860 healthy individuals (mean age 59 ± 16 years; 54% male). These controls were from the general population, Hospital staff, or spouses of the patients, and did not have symptoms of PD or any other neurodegenerative disorder. However, they were not subjected to a detailed examination by a specialist on movement disorders to exclude the existence of asymptomatic PD. The cohort from Asturias was composed by 777 patients (mean age at diagnosis 58 ± 13 years) and 650 controls (58 ± 18 years), and the cohort from Navarra consisted on 250 patients (mean age at diagnosis 57 ± 12) and 210 controls (72 ± 9 years) (Table I). Patients and controls were Spanish Caucasians and signed an informed consent to participate in the study, approved by the Ethical Committee of Hospital Central Asturias.

Analysis of miRNA-133a-1, -133a-2, and -133b Genes

Genomic DNA was obtained from 10 ml of blood, and 350–450 nucleotides of the miR-133a-1, 133a-2, and 133b genes were polymerase chain reaction (PCR)-amplified with primers derived from the corresponding sequences in the *Ensembl* database (www.ensembl.org) (Supplementary Table). The genetic analysis was performed in two steps: search for DNA variants and genotyping of specific variants. To search for DNA variants the PCR fragments from 350 patients and 200 controls (randomly chosen from total patients and controls) were analyzed through denaturing high performance liquid chromatography (DHPLC) in a Varian Helix system, and eluted with a linear binary gradient created with buffers Varian Helix A (triethylammonium amine—TEAA) and B (TEAA + 25% acetonitrile) (www.varianinc.org). Primers and PCR and DHPLC conditions are available upon request to the corresponding author. To identify the nucleotide changes, fragments with multiple elution peaks were sequenced using BigDye chemistry in an automated ABI3130 capillary system (Applied Biosystems, Foster City, CA).

Genotyping of miR-133 Polymorphisms

To determine the association between three DNA variants in the miR-133a-1, -133a-2, and -133b, we genotyped 575 patients and 650 controls through PCR-RFLP: a PCR-fragment was digested with a restriction enzyme which target sequence was affected by the nucleotide change, followed by electrophoresis on 3% agarose gels to visualize the different alleles. Because we found an statistical trend toward the association of the miRNA-133b polymorphism

TABLE I. Main Characteristics of Patients With PD and Controls From the Populations of Asturias and Navarra

	Patients Asturias (n = 777)	Controls Asturias (n = 650)	Patients Navarra (n = 250)	Controls Navarra (n = 210)
Mean, age (years)	58 ± 13	58 ± 18	57 ± 12	72 ± 9
Age, range (years)	16–91	18–96	17–84	37–98
Male	52%	54%	62%	46%
Early onset PD ($\leq$50 years)	181 (23%)	—	71 (28%)	—
Family history of PD	225 (29%)	—	85 (34%)	—
LRRK2, R1441G/C	19 (2%)	—	7 (2%)	—
LRRK2, G2019S	18 (2%)	—	9 (4%)	—

Patients with a family history of PD were those with at least one or second degree relative also diagnosed with PD. Patients with an age at the onset of symptoms $\leq$50 years had an early onset PD (see http://www.ninds.nih.gov/disorders/parkinsons_disease for the diagnostic criteria and the definition of early and late-onset PD). The frequencies of carriers of the common *LRRK2* mutations are also indicated.

and PD, we extended the genotyping of this variant to a total of 1,027 patients and 860 controls.

For the 133a1 +85 A/C variant the DNA was PCR amplified with primers CAGTGACTGAAGCATTGGTATGATAA (forward) and AAGTTATTTGATTATAATCACA GTCTGCTT (reverse). The reverse primer had a mismatched nucleotide in the penultimate position (T instead of A) to introduce and *AflII* site (CTTAAG) on fragments with +85 C. After digestion with this enzyme and electrophoresis on agarose gels, alleles were visualized as fragments of 360 bp (A) or 330 + 30 bp (C). The 133a2 −191 G/A polymorphism was genotyped by digestion with *MspI* (CCGG) of a PCR fragment generated with primers 133a2 F and R (Supplementary Table). In addition to two constant fragments of 55 and 35 bp, alleles were visualized as bands of 354 bp (A) and 212 + 142 bp (G). The 133b −90 ins/del A was genotyped by *MboI* (GATC) digestion of PCR fragments generated with primers 133b F and R (Supplementary Table). Alleles were visualized as fragments of 306 bp (insA) and 231 + 75 bp (delA).

PITX3 Genotyping

We searched for DNA variants in the entire coding and 3′ UTR sequence of *PITX3* (exons 2–4) in 350 patients and 250 controls through single strand conformation analysis (SSCA). Briefly, fragments 300–450 bp were amplified (see the primers in the Supplementary Table), and subjected to SSCA as reported [García-Castro et al., 2003; Gasser et al., 2007]. Fragments that showed different SSCA patterns were sequenced to determine the nucleotide variation. To determine the frequency of the G32S variant, SSCA of the exon 2 fragment was performed on 1,027 patients and 860 controls.

LRRK2 R1441G and G2019S Genotyping

All the patients were characterized for three *LRRK2* mutations: R1441G and R1441C in exon 31 and G2019S in exon 41 [Mata et al., 2006]. Genomic DNA was PCR-amplified with primers that matched intronic sequences flanking exons 31 and 41 of *LRRK2*, followed by sequencing. Primers and PCR and sequencing conditions for the analysis for these mutations are available upon request to the corresponding author.

Statistical Analysis

Allele and genotype frequencies in patients and controls were compared through a Chi-squared test. For those comparisons with any expected frequency below 1, we used the Yate's correction. The Chi-squared was also used to analyze the deviation from the Hardy–Weinberg equilibrium of the genotype frequencies. The ANOVA was used to compare the mean age between the groups. All statistical analyses were performed with the SPSS statistical package (v.11.0). A $P < 0.01$ was considered as statistically significant.

RESULTS

MiRNA-133 Variation

DHPLC analysis of 350 PD-patients and 200 controls showed multiple elution peaks for the 133a-1, 133a-2, and 133b PCR-fragments. We found two new (non previously reported) polymorphisms: −90 ins/del A in 133b and +85 A/C in 133a-1. In the 133a-2 gene we found four previously reported single nucleotide polymorphisms (SNPs): −191 G/A (SNP rs45547937), −171G/A (rs13040566), −88 G/A (rs13040413), and +78 T/C (rs6062251). We sequenced 30 patients and 30 controls and found that they were homozygous for the −191/−171/−88 polymorphisms (−191AA/−171AA/−88AA, or −191GG/−171GG/−88GG), or heterozygous for these SNPs. This suggested that the three 133a-2 SNPs were in complete linkage disequilibrium (LD), defining two haplotypes: −191A/−171A/−88A and −191G/-171G/-88G. We found +78 T/C heterozygotes among individuals homozygotes for the −191G/−171G/−88G haplotype, but all the −191AA/−171AA/−88AA were also +78CC.

MiRNA-133 Genotype Frequencies

We genotyped three nucleotide variants in the miR-133a-1, 133a-2 (rs45547937), and 133b in 575 patients and 650 controls. Allele and genotype frequencies for the 133a-1 and 133a-2 SNPs did not differ between PD-patients and controls. Homozygotes for the miR-133b −90 delA were more frequent among the patients (6/575, 1% vs. 0/650; $P = 0.03$) (Table II). This suggested that homozygosity for −90 delA could be a risk factor for PD. We extended the analysis of this variant to a total of 1,027 patients and 860 controls (including patients/controls from a second population) and we found two healthy controls (aged 66 an 78 years) who were delA-homozygotes. We evaluated these controls to exclude they had symptoms of PD. In addition, they did not have a family history of PD. Although the frequency of −90 delA/delA was higher among the patients, the

TABLE II. Genotype Frequencies for the Three miR-133 Polymorphisms in Patients and Controls

	PD (n = 575)	Controls (n = 650)
miR-133a-1: +85 A/C		
AA	0	0
AC	29 (5%)	29 (4%)
CC	546 (95%)	621 (96%)
miR-133a-2: −191 A/G		
AA	29 (5%)	33 (5%)
AG	178 (31%)	218 (34%)
GG	368 (64%)	399 (61%)
miR133b: −90 ins/delA[a]		
ins/ins	488 (85%)	587 (90%)
ins/del	81 (14%)	63 (10%)
del/del	6 (1%)	0
PITX3: c.285 T/C, Rs2281983		
TT	215 (37%)	227 (35%)
TC	274 (48%)	315 (48%)
CC	86 (15%)	108 (17%)

Patients with a family history of PD were those with at least one or second degree relative also diagnosed with PD. No significant difference for the genotype frequencies was found between younger (<50 years) and older controls.[a]$P = 0.03$ (Yate's chi-squared), −9 del/delA PD versus total controls.[a]The nucleotide variants were numbered according to the mature miRNA sequences, with −1 as the first nucleotide 5′ upstream and +1 as the first nucleotide 3′ downstream.

difference did not reach statistical significance ($P = 0.03$) (Table III).

To exclude that incomplete restriction enzyme digestion could result mistyping of some delA homozygotes (that should be genotyped as heterozygotes), we confirmed the genotype for all the delA/insA patients and controls by DHPLC analysis, obtaining a two elution peak that confirmed the heterozygosisty for all these samples.

All the patients were genotyped for three *LRRK2* mutations associated with PD: R1441G, R1441C, and G2019S were found in 4% of the patients from Asturias and in 6% of the patients from Navarra. In Table III we summarized the 133b frequencies in patients with and without these *LRRK2* mutations.

PITX3 Variation

We searched for *PITX3* variants in a total of 350 patients and 250 controls. The full coding and 3′ UTR sequence was amplified with primers that matched the intronic flanking regions, and subjected to SSCA. Fragments from exons 2 and 3 showed heterogeneous SSCA patterns, and after sequencing we characterized five nucleotide variants: c.94G>T (exon 2), G32>S, IVS +6 bp C>T (intron 2), IVS +70 bp G>T (intron 3), IVS +71 bp C>T (intron 3), and c.285 T/C (SNP rs2281983; exon 3, silent for I 95). The three intronic nucleotide changes were new and only found in one patient and one control. SNP rs2281983 had been previously described and allele and genotype frequencies did not differ between patients and controls, suggesting that this variant did not contribute to PD risk. In addition, rs2281983 allele and genotype frequencies did not differ between early-onset and late-onset patients, or between patients with and without a family history of PD (data not shown).

The exon 2 c.93G>T was a missense change (G32S) found in only one of the 1,027 patients (a 52 years old female, negative for the two *LRRK2* mutations, and without relatives affected by PD). Its absence among the 860 controls and the fact that 32G was conserved among species suggests a contribution of this *PITX3* change to PD in this patient, although functional studies should be necessary to confirm a functional effect.

DISCUSSION

Micro RNA 133b and pitx3 have been involved in dopamine neurons survival in mice. MiRNA-133b was specifically expressed in midbrain DNs and was deficient in midbrain tissue from patients with PD [Kim et al., 2007]. MiRNA-133b binds to the 3′ UTR of pitx3, a transcription factor that regulates the maturation and function of midbrain DNs. Pitx3 in turn binds to the miRNA-133b promoter, enhancing its expression. This negative feedback circuit could be essential to maintain DNs survival, and its deregulation could result in behaviors related with dopaminergic loss, such as locomotion [Kosik, 2006; Hébert and De Strooper, 2007; Kim et al., 2007]. The human genes that encode miRNA-133b and *PITX3* are thus candidates to modify the risk for PD. In this report we searched for DNA variants in the three miRNA-133 and the *PITX3* genes, and analyzed the association between these gene variants and PD.

TABLE III. Genotype Frequencies for the 133b Polymorphism in Patients and Controls From Total PD Patients and Controls, and the Two Spanish Populations Studied (Asturias and Navarra)

	133b −90 ins/ins	133b −90 ins/del	133b −90 del/del
Asturias[a]			
Controls (n = 650)	574 (88%)	76 (12%)	0
Patients (n = 777)	679 (87%)	91 (12%)	7 (1%)
Mean-onset age	58 ± 13	58 ± 13	55 ± 12
Early-onset PD (n = 181)	154 (85%)	25 (14%)	2 (1%)
Navarra[b]			
Controls (n = 210)	187 (89%)	21 (10%)	2 (1%)
Patients (n = 250)	226 (90%)	23 (9%)	1 (0.4%)
Mean-onset age	57 ± 12	57 ± 16	45 and 58
Early-onset PD (n = 71)	62 (87%)	8 (12%)	1 (1%)
Total[c]			
Controls (n = 860)	761 (88%)	97 (11%)	2 (<1%)
Patients (n = 1,027)	905 (88%)	114 (11%)	8 (<1%)
Mean-onset age	58 ± 12	57 ± 13	55 ± 13
Early-onset PD (n = 252)	216 (86%)	33 (13%)	3 (1%)
LRRK2			
R1441G/C G2019S (n = 53)	49 (92%)	3 (6%)	1 (2%)
No *LRRK2*			
Mutation (n = 974)	856 (88%)	111 (11%)	7 (1%)

[a] $P = 0.01$ ($P = 0.04$, Yate's).
[b] The two −90 delA/delA controls were 66 and 78 years old.
[c] Total: $P = 0.03$ ($P = 0.07$, Yate's).

We first searched for DNA variants in PD patients and healthy controls, and found several nucleotide changes in *PITX3* and miRNA-133b, 133a-1, and 133a-2. We used DHPLC and SSCA to analyze the variation in PCR-fragments, two indirect techniques with a low rate for false negatives [Buyse et al., 2000; García-Castro et al., 2003; Gasser et al., 2007]. Thus, we cannot exclude that some DNA variants in the four genes were not identified in our study. We compared the allele and genotype frequencies for three miRNA-133 variants between PD patients and controls. Allele and genotype frequencies for two miRNA-133a-1 (+85 A/C) and 133a-2 (rs45547937) polymorphisms did not differ between the two groups. We cannot exclude an association between the three 133a-2 SNPs (rs13040566, rs13040413, and rs6062251) not genotyped in our patients and controls, although this is unlikely for the −171 G/A and −88 G/A that were in complete linkage disequilibrium with −191 G/A.

In a first comparison of 575 patients and 650 controls, homozygotes for the rare miRNA-133b −90 delA were significantly more frequent among the patients. However, this difference was based on the absence of −90 delA/delA among the controls. We extended the analysis to a second set of 452 patients and 210 controls, and we found also two −90 delA homozygotes among the controls. These homozygous controls were 66 and 78 years old, above the mean age of delA/delA patients (55 years; range 35–66) and they did not have symptoms of PD. Thus, although we found a higher frequency of the −90 delA/delA among the patients the difference with the controls was not significant, and this suggested that miRNA-133b variants did not contribute to the risk for PD.

Our search for *PITX3* variants identified five nucleotide variants. One was a reported SNP in exon 3 (rs2281983, silent for I 95I), and allele and genotype frequencies did not differ between patients and controls. Three intronic *PITX3* nucleotide changes were found in only one patient and one control, and a missense change in exon 2 (c.94 G>T, G32S) was found in only one patient and none of the controls. We determined the presence of this change in a total of 1,027 patients and 860 controls, and we did not find the rare c.94T in the controls. This, in addition to the conservation of 32G among species, suggested that 32S could be associated with PD in this patient.

Two recent reports described a positive associations between a SNP (rs3758549) in the *PITX3* promoter and PD [Fuchs et al., 2009; Haubenberger et al., 2009]. However, a third study failed to replicate this association [Bergman et al., 2008]. A study involving 265 patients and 210 controls reported an association between SNP rs2281983 and PD, and the association was more robust among early onset patients [Le et al., 2009]. In our population, allele and genotype frequencies for this *PITX3* polymorphism did not differ between patients and controls, or between early-onset and late-onset patients.

Finally, several genome wide association (GWA) studies have identified a number of SNPs significantly associated to PD [Fung et al., 2006; Gao et al., 2009; Latourelle et al., 2009; Maraganore et al., 2005]. Two recent reports confirmed the association to known genes (i.e., *SNCA* and *MAPT*), and identified two new risk regions on 1q32 and 4p15 [Satake et al., 2009; Simón-Sánchez et al., 2009]. Consistent with our results, none of these GWA studies identified the regions containing the three miRNA-133 and the *PITX3* genes associated with PD.

In conclusion, we report a search for DNA variants in the miRNA -133 and *PITX3* genes in PD patients. We did not find significant associations between these genes variants and PD-risk. Overall, our data indicate that mutations/polymorphisms in these genes involved in dopaminergic neurons survival did not contribute to the risk of suffering PD.

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REFERENCES

Bartel DP. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116(2):281–297.

Bergman O, Håkansson A, Westberg L, Nordenström K, Carmine Belin A, Sydow O, Olson L, Holmberg B, Eriksson E, Nissbrandt H. 2010. PITX3 polymorphism is associated with early onset Parkinson's disease. Neurobiol Aging 31(1):114–117.

Buyse IM, Fang P, Hoon KT, Amir RE, Zoghbi HY, Roa BB. 2000. Diagnostic testing for Rett syndrome by DHPLC and direct sequencing analysis of the MECP2 gene: Identification of several novel mutations and polymorphisms. Am J Hum Genet 67(6):1428–1436.

Clarimon J, Xiromerisiou G, Eerola J, Gourbali V, Hellström O, Dardiotis E, Peuralinna T, Papadimitriou A, Hadjigeorgiou GM, Tienari PJ, Singleton AB. 2005. Lack of evidence for a genetic association between FGF20 and Parkinson's disease in Finnish and Greek patients. BMC Neurol 5(June 209): 11.

Duan R, Pak C, Jin P. 2007. Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. Hum Mol Genet 16(9):1124–1131.

Fuchs J, Mueller JC, Lichtner P, Schulte C, Munz M, Berg D, Wüllner U, Illig T, Sharma M, Gasser T. 2009. The transcription factor PITX3 is associated with sporadic Parkinson's disease. Neurobiol Aging 30(5): 731–738.

Fung HC, Scholz S, Matarin M, Simón-Sánchez J, Hernandez D, Britton A, Gibbs JR, Langefeld C, Stiegert ML, Schymick J, Okun MS, Mandel RJ, Fernandez HH, Foote KD, Rodríguez RL, Peckham E, De Vrieze FW, Gwinn-Hardy K, Hardy JA, Singleton A. 2006. Genome-wide genotyping in Parkinson's disease and neurologically normal controls: First stage analysis and public release of data. Lancet Neurol 5(11):911–916.

Gao X, Martin ER, Liu Y, Mayhew G, Vance JM, Scott WK. 2009. Genome-wide linkage screen in familial Parkinson disease identifies loci on chromosomes 3 and 18. Am J Hum Genet 84(4):499–504.

García-Castro M, Reguero JR, Batalla A, Díaz-Molina B, González P, Alvarez V, Cortina A, Cubero GI, Coto E. 2003. Hypertrophic cardiomyopathy: Low frequency of mutations in the beta-myosin heavy chain (MYH7) and cardiac troponin T (TNNT2) genes among Spanish patients. Clin Chem 49(8):1279–1285.

Gasser RB, Hu M, Chilton NB, Campbell BE, Jex AJ, Otranto D, Cafarchia C, Beveridge I, Zhu X. 2007. Single-strand conformation polymorphism (SSCP) for the analysis of genetic variation. Nature Protoc 1(6): 3121–3128.

Haubenberger D, Reinthaler E, Mueller JC, Pirker W, Katzenschlager R, Froehlich R, Bruecke T, Daniel G, Auff E, Zimprich A. 2009. Association

of transcription factor polymorphisms PITX3 and EN1 with Parkinson's disease. Neurobiol Aging April 2 [Epub ahead of print].

Hébert SS, De Strooper B. 2007. MiRNAs in neurodegeneration. Science 317(5842):1179–1180.

Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, Hannon G, Abeliovich A. 2007. A MicroRNA feedback circuit in midbrain dopamine neurons. Science 317(5842):1220–1224.

Kosik KS. 2006. The neuronal microRNA system. Nat Rev Neurosci 7(12):1–4.

Latourelle JC, Pankratz N, Dumitriu A, Wilk JB, Goldwurm S, Pezzoli G, Mariani CB, DeStefano AL, Halter C, Gusella JF, Nichols WC, Myers RH, Foroud T, PROGENI Investigators, Coordinators and Molecular Genetic Laboratories, GenePD Investigators, Coordinators and Molecular Genetic Laboratories. 2009. Genomewide association study for onset age in Parkinson disease. BMC Med Genet 10:(September 22): 98.

Le W, Nguyen D, Lin XW, Rawal P, Huang M, Ding Y, Xie W, Deng H, Jankovic J. 2009. Transcription factor PITX3 gene in Parkinson's disease. Neurobiol Aging April 24 [Epub ahead of print].

Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. 2004. MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23(20):4051–4060.

Li W, Duan R, Kooy F, Sherman SL, Zhou W, Jin P. 2009. Germline mutation of microRNA-125a is associated with breast cancer. J Med Genet 46(5):358–360.

Maraganore DM, de Andrade M, Lesnick TG, Strain KJ, Farrer MJ, Rocca WA, Pant PV, Frazer KA, Cox DR, Ballinger DG. 2005. High-resolution whole-genome association study of Parkinson disease. Am J Hum Genet 77(5):685–693.

Mata IF, Ross OA, Kachergus J, Huerta C, Ribacoba R, Moris G, Blazquez M, Guisasola LM, Salvador C, Martinez C, Farrer M, Alvarez V. 2006. LRRK2 mutations are a common cause of Parkinson's disease in Spain. Eur J Neurol 13(4):391–394.

Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T. 2009. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 41(12):1303–1307.

Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Krüger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T. 2009. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41(12): 1308–1312.

Valencia-Sánchez MA, Liu J, Hannon GJ, Parker R. 2006. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes Dev 20(5):515–524.

Wang G, van der Walt JM, Mayhew G, Li YJ, Züchner S, Scott WK, Martin ER, Vance JM. 2008. Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. Am J Hum Genet 82(2):283–289.

Wider C, Dachsel JC, Soto AI, Heckman MG, Diehl NN, Yue M, Lincoln S, Aasly JO, Haugarvoll K, Trojanowski JQ, Papapetropoulos S, Mash D, Rajput A, Rajput AH, Gibson JM, Lynch T, Dickson DW, Uitti RJ, Wszolek ZK, Farrer MJ, Ross OA. 2009. FGF20 and Parkinson's disease: No evidence of association or pathogenicity via alpha-synuclein expression. Mov Disord 24(3):455–459.