Supplementary Material

Lack of Association between *DNMT3B* Polymorphisms and Sporadic Parkinson's Disease in a Han Chinese Population

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Materials and Methods

Participants

The diagnosis of PD patients was based on the UK Brain Bank criteria [1]. Controls were recruited from a community, and examined by a movement disorder specialist for exclusion of neurodegenerative diseases, malignant tumors, and auto-immune diseases. All the PD cases and controls were Han Chinese and signed consent forms. This study was approved by the Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine [2011 No.13].

Genotyping

Genomic DNA was extracted from the peripheral venous blood of PD patients and controls using the classical phenol-chloroform-isopropyl alcohol method [2]. Primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by BioSune biotechnology (Shanghai) Co., Ltd., Shanghai, China. Sequencing results were analyzed with SeqMan in DNASTAR Lasergene package, version 7.1.0 (DNASTAR, Inc.). Details of the primers are available in Table S5.

Statistics

Student *t*-test was used to compare age between PD and control groups. Gender differences were assessed using the χ^2 test. Hardy-Weinberg equilibrium was tested by the χ^2 test or Fisher's exact test. Risk analysis was carried out using a logistic regression model. Odds ratios were calculated with 95% confidence intervals for each SNP according to allelic association, the dominant model, and the recessive model. The statistical analyses were conducted using R, version

3.4.1 (R Core Team, https://www.R-project.org), and RStudio Desktop, version 1.0.153, (RStudio, Inc. Boston, MA). Logistic regression was performed in SPSS, version 21.0.0 (IBM Corporation). The power effect was calculated using PS Power and Sample Size Calculations, version 3.1.2 (Dupont WD, Plummer) [3, 4]. Linkage disequilibrium was analyzed by HaploView, version 4.2 (Daly Lab at the Broad Institute, Cambridge, MA).

Meta-analysis

We searched PubMed, Web of Science, Cochrane Library, and EMBASE, and found only one study investigating the association between rs2424913 on *DNMT3B* and PD in an Asian population. STATA SE, version 12.0 (StataCorp) was applied in the meta-analysis. A fixed effect model was chosen because no heterogeneity was found (I^2 <50%). Forest plots were drawn in Review Manager (RevMan), version 5.3.5 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen).

Table S1. Demographic information in this study.

	PD (n = 249)	Control (n = 239)	*P*
Age (years, mean ± SD)	63.97±11.02	64.75±10.72	0.425
Female/male	124/125	115/124	0.779
Onset age (years, mean ± SD)	56.29±11.12	-	-

PD, Parkinson's disease; SD, standard deviation.

Table S2. Association of candidate SNPs with PD risk.

Gene	SNP_ID	HWE p	PD MAF (%)	HC MAF (%)	Power	Allele	Additive model			Dominant Model			Recessive model					
						minor allele	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI
RAD51B	rs34094401	-	0.00	0.00	-	G	-	-	-	-	-	-	-	-	-	-	-	-
CPXM1	rs41309351	-	0.00	0.00	-	A	-	-	-	-	-	-	-	-	-	-	-	-
SERPINA1	rs141620200	-	0.00	0.00	-	A	-	-	-	-	-	-	-	-	-	-	-	-
MPHOSPH10	rs143555311	-	0.00	0.00	-	G	-	-	-	-	-	-	-	-	-	-	-	-
MPHOSPH10	rs9941620	0.886	27.11	30.75	0.23	G	0.200	0.83	0.62-1.11	0.218	0.84	0.64-1.11	0.239	0.80	0.55-1.16	0.519	0.78	0.39-1.54
DNMT3B	rs2424913	1.000	0.40	0.84	0.07	C	0.443	0.48	0.04-3.35	0.394	0.48	0.09-2.62	0.443	0.48	0.04-3.36	1.000	0.00	-.
DNMT3B	rs75826787	0.915	5.22	4.81	0.06	T	0.884	1.09	0.59-2.03	0.799	1.10	0.61-1.98	0.880	1.10	0.58-2.08	1.000	0.00	-.

HWE p, *P* value of Hardy-Weinberg equilibrium; MAF, minor allele frequency of the SNP; PD, Parkinson's disease; HC, healthy control; OR, odds ratio; 95% CI, 95% confidence interval.

Table S3. Linkage disequilibrium (LD) of two SNPs on chromosome 20.

Locus 1	Locus 2	D'	LOD	r^2	95% CI	Dist (bp)
rs75826787	rs2424913	1	0.13	0	0.04-0.97	93

D', normalized coefficient of LD between the two loci; LOD, log of the likelihood odds ratio, a measure of confidence in the value of D'; r^2, correlation coefficient between the loci; 95% CI, confidence interval of D'; Dist, distance (in base pairs) between the loci.

Table S4. Meta-analysis of SNPs of rs2424913 *DNMT3B* in a Chinese Population.

GENE	SNP_ID	Power	gender	Allele				Dominant Model			
				I^2	*P*	OR	95% CI	I^2	*P*	OR	95% CI
DNMT3B	rs2424913	0.09	total	0	0.419	0.65	0.23-1.84	0	0.420	0.65	0.23-1.84
		0.17	female	0	0.321	0.50	0.12-1.98	0	0.319	0.49	0.12-1.98
		0.05	male	0	0.968	0.97	0.20-4.81	0	0.968	0.97	0.19-4.83

OR, odds ratio; 95% CI, 95% confidence interval.

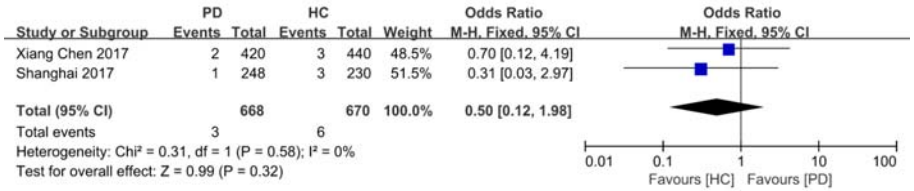
Table S5. Primers amplifying the candidate SNPs.

Gene	SNP	Primer type	Primer sequences (5'→3')	Product (bp)
RAD51B	rs34094401	Forward	GCTTTGAGGCCAGAATTCAGTGTT	1216
		Reverse	TGCTGTTTTAAGCTACTAAACTGTGAG	
CPXM1	rs41309351	Forward	GGGACACTTCAGAGTGGGAACA	889
		Reverse	TTCTGCTCCTGATGCAGTTCCT	
MPHOSPH10	rs143555311 & rs9941620	Forward	GTTCCAGTTGGTCTTATAATTTTGTGGT	983
		Reverse	CCGCATCATCTGGTAAAGCAAA	
SERPINA1	rs141620200	Forward	CTTTGCTGTTTGACCACGTCCC	694
		Reverse	TAAACGGTTGTCACTGGGCACT	
DNMT3B	rs2424913 & rs75826787	Forward	CATTTCAGCTGGCCATGTCTGTCT	634
		Reverse	AGGGAAAGCTTCCATTCCCAACA	

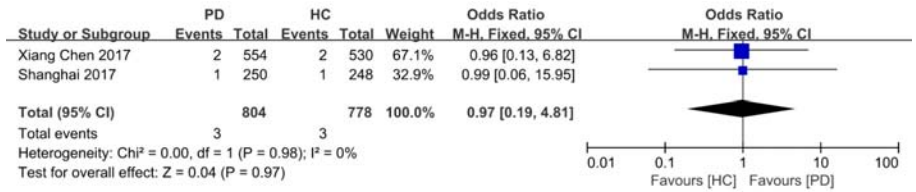
Rs143555311 and rs9941620 shared amplifying primers; Rs75826787 and rs2424913 also shared amplifying primers.

A Allelic association in total:

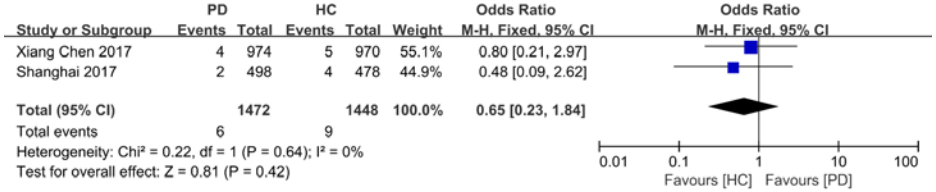
B Allelic association in females:



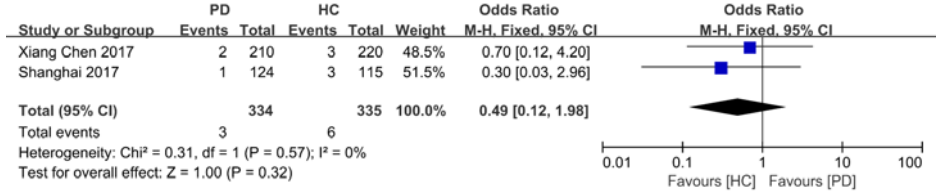
C Allelic association in males:



D Dominant model of genotype in total:



E Dominant model of genotype in females:



F Dominant model of genotype in males:

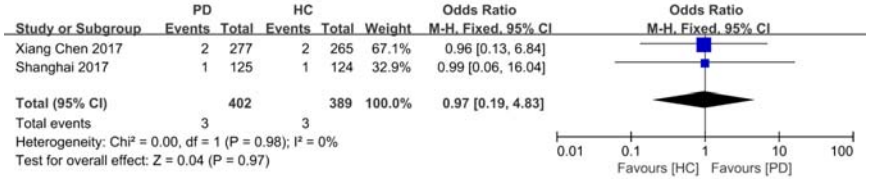


Fig. S1. Meta-analysis of rs2424913 *DNMT3B*. A fixed effect model was applied. Xiang Chen, the study of Xiang Chen et al.[5]; Shanghai, our study; OR, odds ratio; 95% CI, 95% confidence interval.

References

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[2] Ahmad NN, Cu-Unjieng AB, Donoso LA. Modification of standard proteinase K/phenol method for DNA isolation to improve yield and purity from frozen blood. J Med Genet 1995, 32: 129-130.

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[4] Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. Control Clin Trials 1990, 11: 116-128.

[5] Chen X, Xiao Y, Wei L, Wu Y, Lu J, Guo W, et al. Association of DNMT3b gene variants with sporadic Parkinson's disease in a Chinese Han population. J Gene Med 2017, 19: 360-365