Methods

Study population

A total of 90 PD patients from the Movement Disorder Clinic at the Department of Neurology of Ruijin Hospital (urban area) and the First People's Hospital of Tonglu (rural area) from September 2016 to September 2017 were recruited for this hospital-based case-control study. Healthy spouses with whom they had been living in the same household for at least 20 years were recruited as controls. All PD patients were examined by at least two movement disorder specialists at the Department of Neurology of Ruijin Hospital. Inclusion criteria were as follows: (1) diagnosed as idiopathic PD according to 2015 MDS clinical diagnostic criteria for PD [1]; (2) no family history of PD and (3) no history of other significant neurological disorders, including cerebral stroke, epilepsy, head trauma and other concomitant disease potentially associated with PD and familial history of any kind of cognitive/behavioral abnormality. Healthy controls were assessed by at least two neurologists and were recruited from healthy subjects without significant disease symptoms, especially malignant tumor and significant neurological disorders, such as cerebral stroke, head trauma and essential tremor. Clinical features of PD patients including disease duration, the Hoehn and Yahr stage (H&Y stage), Unified Parkinson's Disease Rating Scale (UPDRS), Nonmotor Symptoms Questionnaire for PD (NMS), Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) scores were recorded [2]. The study protocol was approved by the Research Ethics Committee, Ruijin Hospital, Shanghai Jiaotong University School of Medicine (No. 2017-8). All participants signed an informed consent. Clinical data were collected through face-to-face interviews with movement disorder specialists.

Measurement of pesticide levels

The serum levels of 19 pesticides including 16 OCPs (α-hexachlorocyclohexane (α-HCH), β-HCH, γ-HCH, δ-HCH, propanil, vinclozolin, heptachlor, Aldrin, dieldrin, endosulfan, hexachlorobenzene, quintozene, p,p'-DDE, p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT), p,p'-DDT) and 3 OPs (parathion-methyl, methidathion, phosaloneand) which were ever wildly used in China were measured by gas chromatography/mass spectrometry (GC-MS) [3]. Briefly, 500 mg of urea was added to 1 ml of serum, followed by sonication in the ultrasonic water bath for 20 min. The sample was then pre-concentrated by passage through an Oasis HLB column (Waters, Milford, MA, USA) pretreated with methyl alcohol. The analyte was eluted in methylene chloride and acetone, and evaporated to dryness with a Termovap sample concentrator (ANPEL, USA). The residue was resuspended in 100 μl of methylene chloride and detected with a Model TSQ8000 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The limit of detection (LOD) for the pesticides was appropriately 0.1 ng/ml. For quality control, five blood samples in triplicate were spiked with a mixed standard of OCPs at 5 and 50 ng/ml. The average recoveries of samples exceeded 95%. PD patients and control samples were analyzed in the same batch. To maintain accuracy, a quality check sample was included in each set of samples. The results were adjusted for the total serum lipid content and were reported as nanogram per gram lipid. Total lipids in plasma were calculated as previously described [4].

Genetic analysis

Genomic DNA was prepared from peripheral blood leukocytes by the conventional phenol/chloroform extraction method. We used MassARRAY Assay Design 3.0 software (Sequenom, San Diego, CA, USA) to design Multiplexed single nucleotide

polymorphism (SNP) MassEXTEND assay. A total of 10 SNPs were analyzed in this study. Of which, 5 SNPs were associated with the risk of PD, including rs11931074 and rs3775423 in *SNCA* gene, rs16940758 and rs2435211 located in microtubule-associated protein tau (*MAPT*) gene, rs733731 in peptidoglycan recognition protein 2 (*PGLYRP2)* gene [5-7]. The other 5 SNPs were located in genes related to pesticides transportation and metabolization, including rs1045642 in ATP-binding cassette, sub-family B, member 1 (*ABCB1*) rs12829185 in *NOS1*, rs4646903 in cytochrome P450, family 1, subfamily A, polypeptide 1 (*CYP1A1*), rs1056836 in *CYP1B1* and rs7260538 in *CYP2B6* gene [8-11]. SNP genotyping was performed by the chip-based matrix-assisted laser desorption ionization time-aof-flight mass spectrometry method using the Mass-ARRAY RS1000 platform (Sequenom, San Diego, USA) [12]. The corresponding primers used for each SNP are listed in Table e-5. Typer 4.0 software (Sequenom) was used for data analysis. Genotyping was performed by investigators who were blinded to the clinical status of the subjects.

Cell culture and Cell viability

Human neuroblastoma SH-SY5Y cell line was obtained from the American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified Eagle medium, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution in a humidified 5% CO2 atmosphere at 37°C. Cell viability was determined by the Cell Counting Kit 8 (CCK-8) assay. Cells were incubated for 24 h in a 96-well plate at 5.0 × 103 cells/well. After different treatments, each well was supplemented with 10 μl CCK-8. After 4 h of incubation at 37°C, the absorbance at 570 nm was measured at 570 nm with a microplate reader (BioTek, Winooski, VT, USA).

Measurement of reactive oxidative stress

Reactive oxidative stress (ROS) was detected with chloromethyl derivative of

dichlorodihydrofluorescein diacetate (CM-H2DCFDA, Invitrogen, USA), which are readily taken up by cells. The probe was diluted to a final concentration of 2 μM with dimethylsulfoxide (DMSO). After different treatments, cells were incubated with CM-H2DCFDA at 37°C for 30 min before detection by flow cytometer at excitation and emission at wavelengths of 485 nm and 535 nm, respectively.

Measurement of mitochondrial membrane potential (ΔΨm)

Mitochondrial membrane potential (ΔΨm) was measured using fluorescent probe JC-10 (Yeasen Technology, China). At a high potential, JC-10 polymers emit red fluorescence, while at low potential, JC-10 exists as a monomer and is detectable based on green fluorescence. After different treatments, cells were incubated with10 μM JC-10 probe at 37°C for 30 min before detection. The fluorescence intensity of JC-10 was measured by flow cytometer with excitation and emission wavelengths of 490 and 530 nm to detect monomers of JC-10 and excitation and emission wavelengths of 525 and 590 nm to detect polymers of JC-10 respectively. ΔΨm was calculated by normalizing red fluorescence to green fluorescence signal intensity values.

Western blot analysis

Cells with different treatments were lysed in radioimmunoprecipitation lysis buffer (50 mM Tris–HCl [pH 8.0], 1% NP-40, 0.5% sodium deoxycholate, 150 mM NaCl, 0.1% sodium-dodecyl sulfate containing protease inhibitor cocktails and 1 mM phenyl-methylsulfonyl fluoride). Proteins were separated by polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% bovine serum albumin for 2 h at room temperature and incubated overnight at 4°C with primary antibodies (β-actin, Sigma; α-synuclein, BD). After three washes in Tris-buffered saline with 0.1% Tween-20 (TBST), the membrane was incubated with appropriate horseradish peroxidase-conjugated

secondary antibodies (Jackson Laboratories) at 25°C for 1 h. After being washed three times with TBST, protein bands were visualized using an enhanced chemiluminescence detection system.

Statistical analysis

All statistical analysis were conducted with SPSS software Version 21.0 (SPSS Inc., USA), STATA (Version 15) and GraphPad Prism 5.0 software. The Hardy–Weinberg equilibrium χ^2 test was used to assess the goodness of fit for each SNP data. The χ^2 test was used to analyze categorical variables including sex, genotype, allele distribution and the detection rate of pesticides. For samples with non-detectable pesticides levels, we used a value equal to half of the LOD (0.05 ng/mL) as input [13]. The Mann-Whitney U test was used to evaluate the differences in pesticide levels between PD patients and controls. Due to shrew distribution (not normally distributed), the levels of pesticides were divided into tertiles as indicated by the distribution of concentrations of pesticides in controls [14, 15]. Logistic regression analysis was performed to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) for the association between serum pesticide levels and PD diagnosis. Multiplicative interactions between serum levels of pesticides and genotypes were tested using a logistic regression model with covariate adjustment, such as sex, age and region. One-way ANOVA and post hoc corrections (Bonferroni) was used for comparisons of clinical characteristics in PD patients with different serum levels of pesticides and multiple experimental conditions. Multiple stepwise linear regression was used to assess the precise association between the pesticides levels and clinical characteristics. All analysis were 2-tailed, and the level of statistical significance was set at $P < 0.05$.

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Supplementary Tables:

Table e-1. Demographic and clinical characteristics of the participants

	Total (n=180)			Urban area (n=88)			Rural area (n=92)		
	PD (n=90)	Con (n=90)	P value	PD (n=44)	Con (n=44)	P value	PD (n=46)	Con (n=46)	P value
Age (years)	65.76±9.90	64.23±9.14	0.3	64.59±8.72	63.09±9.25	0.4	66.87±10.88	65.33±9.00	0.5
Sex (%)			0.02*			0.1			0.1
Male	53 (58.9)	37 (41.1)		26 (59.1)	18(40.9)		27 (58.7)	19 (41.3)	
Female	37 (41.1)	53 (58.9)		18 (40.9)	26(59.1)		19 (41.3)	27 (58.7)	
Age of onset (years)	59.83±10.43	NA		59.39±8.83	NA		60.26±11.84	NA	
Disease duration (years)	5.60±4.48	NA		4.18±3.72	NA		6.96±4.76	NA	
H&Y stage	2.29±0.99	NA		1.93±0.78	NA		2.59±1.12	NA	
UPDRS Part I	4.32±3.03	NA		3.11±2.36	NA		5.48±3.17	NA	
UPDRS Part II	14.00±11.53	NA		10.84±11.27	NA		17.02±11.07	NA	
UPDRS Part III	28.97±18.51	NA		21.20±13.89	NA		36.39±19.45	NA	
UPDRS Part IV	2.79±3.14	NA		1.59±2.14	NA		3.93±3.52	NA	
UPDRS total	49.53±31.97	NA		35.64±20.92	NA		62.83±35.12	NA	
NMS	7.74±4.80	NA		6.30 ± 3.83	NA		9.13±5.26	NA	
HAMD	7.77±8.37	NA		5.61±7.09	NA		9.83±9.03	NA	
HAMA	9.33±8.63	NA		7.14±8.17	NA		11.43±8.61	NA	
MMSE	25.96±4.75	NA		27.66±4.63	NA		24.33±4.31	NA	
MoCA	22.17±5.49	NA		24.48±4.53	NA		19.96±5.45	NA	

Data were shown as mean±SD except for Sex, which were shown as n (%)

Abbreviations: PD, Parkinson's disease; Con, control; H&Y stage, Hoehn and Yahr stage; UPDRS, Unified Parkinson's Disease Rating Scale; NMS, Non-Motor Symptoms; HAMD, Hamilton Depression Scale; HAMA, Hamilton Anxiety Scale; MMSE, Mini Mental State Examination; MoCA, Montreal Cognitive Assessment; SD, standard deviation. UPDRS scores were obtained during the on-phase at the outpatient clinic. *: $P<0.05$.

Table e-2. Detected pesticides in PD patients and controls

Pesticides	Total (n=180)			Urban area (n=88)			Rural area (n=92)		
	PD (n=90)	Con (n=90)	P value	PD (n=44)	Con (n=44)	P value	PD (n=46)	Con (n=46)	P value
α-HCH (%)	46 (51.1)	31 (34.4)	0.02*	21 (47.7)	15 (34.1)	0.2	25 (54.3)	16 (34.8)	0.1
β-HCH (%)	70 (77.8)	57 (63.3)	0.03*	31 (70.5)	24 (54.5)	0.1	39 (84.8)	33 (71.7)	0.1
γ-HCH (%)	58 (64.4)	50 (55.6)	0.2	26 (59.1)	24 (54.5)	0.7	32 (69.6)	26 (56.5)	0.2
δ-HCH (%)	68 (75.6)	57 (63.3)	0.1	35 (79.5)	33 (75.0)	0.6	33 (71.7)	24 (52.2)	0.1
Propanil (%)	88 (97.8)	87 (96.7)	0.7	42 (95.5)	42 (95.5)	1.0	46 (100)	45 (97.8)	0.3
Vinclozolin (%)	86 (95.6)	89 (98.9)	0.2	42 (95.5)	43 (97.7)	0.6	44 (95.7)	46 (100)	0.2
Heptachlor (%)	42 (46.7)	31 (34.4)	0.1	14 (31.8)	18 (40.9)	0.4	28 (60.9)	13 (28.3)	<0.01**
Aldrin (%)	87 (96.7)	80 (88.9)	0.04*	41 (93.2)	37 (84.1)	0.2	46 (100)	43 (93.5)	0.1
Dieldrin (%)	86 (95.6)	85 (94.4)	0.7	41 (93.2)	40 (90.9)	0.7	45 (97.8)	45 (97.8)	1.0
Endosulfan (%)	83 (92.2)	78 (86.7)	0.2	40 (90.9)	38 (86.4)	0.5	43 (93.5)	40 (87.0)	0.3
Hexachlorobenzene (%)	55 (61.1)	52 (57.8)	0.6	17 (38.6)	22 (50.0)	0.3	38 (82.6)	30 (65.2)	0.1
Quintozene (%)	69 (76.7)	64 (71.1)	0.4	29 (65.9)	31 (70.5)	0.6	40 (87.0)	33 (71.7)	0.1
p,p'-DDE (%)	86 (95.6)	76 (84.4)	0.01*	41 (93.2)	39 (88.6)	0.5	45 (97.8)	37 (80.4)	<0.01**
p,p'-DDD (%)	81 (90.0)	76 (84.4)	0.3	41 (93.2)	35 (79.5)	0.1	40 (87.0)	41 (89.1)	0.7
o,p'-DDT (%)	85 (94.4)	81 (90.0)	0.3	41 (93.2)	39 (88.6)	0.5	44 (95.7)	42 (91.3)	0.4
p,p'-DDT (%)	79 (87.8)	74 (82.2)	0.3	37 (84.1)	37 (84.1)	1.0	42 (91.3)	37 (80.4)	0.1
Parathion-methyl (%)	47 (52.2)	41 (45.6)	0.4	24 (54.5)	23 (52.3)	0.8	23 (50.0)	18 (39.1)	0.3
Methidathion (%)	43 (47.8)	36 (40.0)	0.3	19 (43.2)	17 (38.6)	0.7	24 (52.2)	19 (41.3)	0.3
Phosalone (%)	48 (53.3)	50 (55.6)	0.8	26 (59.1)	23 (52.3)	0.5	22 (47.8)	27 (58.7)	0.3

Data were shown n (%)

Abbreviations: PD, Parkinson's disease; Con, control; α-HCH, α-hexachlorocyclohexane; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; δ-HCH, δ-hexachlorocyclohexane; p,p'-DDE, p,p'-dichloro-diphenyldichloroethylene; p,p'-DDD, p,p'-Dichlorodiphenyldichloroethane; o,p'-DDT, o,p'-dichloro-diphenyl-trichloroethane, p,p'-DDT, p,p'-dichloro-diphenyl-trichloroethane. *: P<0.05, **: P<0.01.

Table e-3. Association of the serum levels of pesticides and risk of PD

Categorical levels of pesticides	PD (n=90)	Con (n=90)	OR (95% CI)	P value [a]	OR (95% CI)	P value [b]
α-HCH				<0.01**		0.01*
Tertile 1 (%)	30(33.3)	10(11.1)	1 [Reference]			
Tertile 2 (%)	30(33.3)	35(38.9)	3.6 [1.5-8.8]	<0.01**	2.8 [1.1-7.0]	0.03*
Tertile 3 (%)	30(33.3)	45(50.0)	4.5 [1.9-10.7]	<0.01**	3.9 [1.6-9.5]	<0.01**
β-HCH				0.04*		
Tertile 1 (%)	30(33.3)	17(18.9)	1 [Reference]			
Tertile 2 (%)	30(33.3)	32(35.6)	1.9 [0.9-4.2]	0.1		
Tertile 3 (%)	30(33.3)	41(45.6)	2.7 [1.2-5.9]	0.01*		
γ-HCH				0.2		
Tertile 1 (%)	30(33.3)	19(21.1)	1 [Reference]			
Tertile 2 (%)	30(33.3)	36(37.8)	NA	0.7		
Tertile 3 (%)	30(33.3)	37(41.1)	NA	0.2		
δ-HCH				0.02*		
Tertile 1 (%)	30(33.3)	15(16.7)	1 [Reference]			
Tertile 2 (%)	30(33.3)	31(34.4)	2.1 [0.9-1.1]	0.1		
Tertile 3 (%)	30(33.3)	44(48.9)	3.0 [1.4-6.6]	<0.01**		
Propanil				<0.01**		<0.01**
Tertile 1 (%)	30(33.3)	17(18.9)	1[Reference]			
Tertile 2 (%)	30(33.3)	16(17.8)	0.8 [0.3-2.0]	0.7	1.0 [0.4-2.5]	1.0
Tertile 3 (%)	30(33.3)	57(63.3)	3.3 [1.6-7.1]	<0.01**	3.3 [1.5-7.2]	<0.01**

Heptachlor				0.01*
Tertile 1 (%)	30(33.3)	12(13.3)	1 [Reference]	
Tertile 2 (%)	30(33.3)	34(37.8)	2.8 [1.2-6.4]	0.02*
Tertile 3 (%)	30(33.3)	44(48.9)	3.5 [1.5-8.0]	<0.01**
Dieldrin				0.02*
Tertile 1 (%)	30(33.3)	13(14.4)	1 [Reference]	
Tertile 2 (%)	30(33.3)	33(36.7)	2.5 [1.1-5.8]	0.03*
Tertile 3 (%)	30(33.3)	44(48.9)	3.3[1.5-7.4]	<0.01**
Hexachlorobenzene				0.03*
Tertile 1 (%)	30(33.3)	25(27.8)	1 [Reference]	
Tertile 2 (%)	30(33.3)	18(20.0)	0.7 [0.3-1.6]	0.3
Tertile 3 (%)	30(33.3)	47(52.2)	1.9 [1.0-3.9]	0.1
p,p'-DDE				0.02*
Tertile 1 (%)	30(33.3)	19(21.1)	1 [Reference]	
Tertile 2 (%)	30(33.3)	23(25.6)	1.2 [0.5-2.6]	0.7
Tertile 3 (%)	30(33.3)	48(53.3)	2.7 [1.3-5.6]	0.01*
o,p'-DDT				0.03*
Tertile 1 (%)	30(33.3)	15(16.7)	1 [Reference]	
Tertile 2 (%)	30(33.3)	30(33.3)	1.9 [0.9-4.3]	0.1
Tertile 3 (%)	30(33.3)	45(50.0)	3.0 [1.4-6.5]	<0.01**

Data were shown as n (%)

Abbreviations: PD, Parkinson's disease; Con, control; NA, not available; α-HCH, α-hexachlorocyclohexane; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane, δ-HCH, δ-hexachlorocyclohexane; p,p'-DDE, p,p'-dichloro-diphenyldichloroethylene, o,p'-DDT, o,p'-dichloro-diphenyl-trichloroethane; OR: Odds Ratio; CI: confidence interval; LOD: the limit of detection.[a]: Adjusted by age, gender and region; [b]: Adjusted by age, gender, region and pesticides. *: P<0.05, **: P<0.01.

Table e-4. Genotype and allele distributions of SNPs in PD patients and controls

	Genotypes / Alleles		PD (n=90)	Con (n=90)	P value
SNCA rs11931074	Genotypes (%)	GG	14(15.6)	18(20.0)	0.5
		GT	45(50.0)	37(41.1)	
		TT	31(34.4)	35(38.9)	
	Alleles (%)	G	73(40.6)	73(40.6)	1.0
		T	107(59.4)	107(59.4)	
SNCA rs3775423	Genotypes (%)	CC	14(15.6)	22(24.4)	0.3
		CT	45(50.0)	37(41.1)	
		TT	31(34.4)	31(34.4)	
	Alleles (%)	C	73(40.6)	81(45.0)	0.4
		T	107(59.4)	99(55.0)	
MAPT rs16940758	Genotypes (%)	CC	68(75.6))	67(74.4)	0.4
		CT	17(18.9)	21(23.3)	
		TT	5(5.6)	2(2.2)	
	Alleles (%)	C	153(85.0)	155(86.1)	0.8
		T	27(15.0)	25(13.9)	
MAPT rs2435211	Genotypes (%)	CC	57(63.3)	52(57.8)	0.3
		CT	28(31.1)	36(40.0)	
		TT	5(55.6)	2(2.2)	
	Alleles (%)	C	142(78.9)	140(77.8)	0.8
		T	38(21.1)	40(22.2)	
PGLYRP2 rs733731	Genotypes (%)	CC	32(35.6)	33(36.7)	0.9
		CT	49(54.4)	46(51.1)	
		TT	9(10.0)	11(12.2)	
	Alleles (%)	C	113(62.8)	112 (62.2)	0.9
		T	67(37.2)	68(37.5)	

ABCB1 rs1045642	Genotypes (%)	AA	10(11.1)	16(17.8)	0.3
		AG	39(13.0)	41(45.6)	
		GG	41(45.6)	33(36.7)	
	Alleles (%)	A	59(32.8)	73(40.6)	0.1
		G	121(67.2)	107 (59.4)	
NOS1 rs12829185	Genotypes (%)	CC	47(52.2)	46(51.1)	0.8
		CT	40(44.4)	39(43.3)	
		TT	3(3.3)	5(5.6)	
	Alleles (%)	C	134(74.4)	131 (72.8)	0.7
		T	46(25.6)	49(27.2)	
CYP1A1 rs4646903	Genotypes (%)	AA	43(47.8)	35(38.9)	0.2
		AG	33(36.7)	45(50.0)	
		GG	14(15.6)	10(11.1)	
	Alleles (%)	A	119(66.1)	115 (63.9)	0.7
		G	61(33.9)	65(36.1)	
CYP1B1 rs1056836	Genotypes (%)	GG	60(66.7)	67(74.4)	0.2
		GC	29(32.2)	20(22.2)	
		CC	1(1.1)	3(3.3)	
	Alleles (%)	G	150(83.3)	155 (86.1)	0.5
		C	30(16.7)	25(13.9)	
CYP2B6 rs7260538	Genotypes (%)	TT	10(11.1)	13(14.4)	0.6
		TG	39(43.3)	33(36.7)	
		GG	41(45.6)	44(48.9)	
	Alleles (%)	T	59(32.8)	59(32.8)	1.0
		G	121(67.2)	121 (67.2)	

Data were shown as n (%).

Abbreviations: PD: Parkinson's disease; Con: control; SNCA: α-synuclein; MAPT: aumicrotubule associated protein tau; ABCB1： PGLYRP2: peptidoglycan recognition protein 2; ATP-Binding Cassette, subfamily B, member 1; NOS1: nitric oxide synthase 1; CYP: cytochrome enzyme P450.

Table e-5. Primers for the amplification and single base extension of SNPs genotypes

SNPs	Primer Sequence for amplification (5'-3')	Primer Sequence for single base extension
SNCA rs11931074	F: ACGTTGGATGACAGTCAAATGGCAGCCTTC R: ACGTTGGATGTGCCACTATTTCTTCCTCGG	CTTCCAAATCATAATTCCCT
SNCA rs3775423	F: ACGTTGGATGATGACCAAAGGACTTGGTGC R: ACGTTGGATGAGCTCTTAATGCTGGGCTTG	CAAAGGACTTGGTGCATTTAA
MAPT rs16940758	F: ACGTTGGATGTTCATTTGGAAAGAGAGAGG R: ACGTTGGATGCCTGGGATTCTTACTGTAGC	AGAGAGAGGAAGAGGC
MAPT rs2435211	F: ACGTTGGATGAAGCCTGGGAGTCTTTTAGC R: ACTTGGATGAGGGACCAGCAATGAGTATG	GGCGGCCACATAGTATAGTTGGAAAA
PGLYRP2 rs733731	F: ACGTTGGATGCCATATTCCTTCCCTTCTCG R: ACGTTGGATGATGCTACAGAGTTGGATCCC	GACCTTCCCTTCTCGTACGTCATGT
ABCB1 rs1045642	F: ACGTTGGATGTAGGCAGTGACTCGATGAAG R: ACGTTGGATGTATGGAGACAACAGCCGGGT	CTTTGCTGCCCTCAC
NOS1 rs12829185	F: ACGTTGGATGACCACAAAGGACTCAGCAAC R: ACGTTGGATGGAGAACATGCTGCCGTATTC	AGCAACGGTCAGTCT
CYP1A1 rs4646903	F: ACGTTGGATGCTGAGGTGGGAGAATCGTGT R: ACGTTGGATGAGTGCACTGGTACCATTTTG	GAGAATCGTGTGAGCCC
CYP1B1 rs1056836	F: ACGTTGGATGCAACCAGTGGTCTGTGAATC R: ACGTTGGATGATCACTCTGCTGGTCAGGTC	CTGTGAATCATGACCCA
CYP2B6 rs7260538	F: ACGTTGGATGCAGCTATGCATACTGTTCTG R: ACGTTGGATGGTAAAGGCAGTCTTCTGCTC	GGCATATTTCCAGGTTAAT

Abbreviations: SNCA: α-synuclein; MAPT: aumicrotubule associated protein tau; ABCB1：PGLYRP2: peptidoglycan recognition protein 2; ATP-Binding Cassette, subfamily B, member 1; NOS1: nitric oxide synthase 1; CYP: cytochrome enzyme P450.

Supplementary Figures:

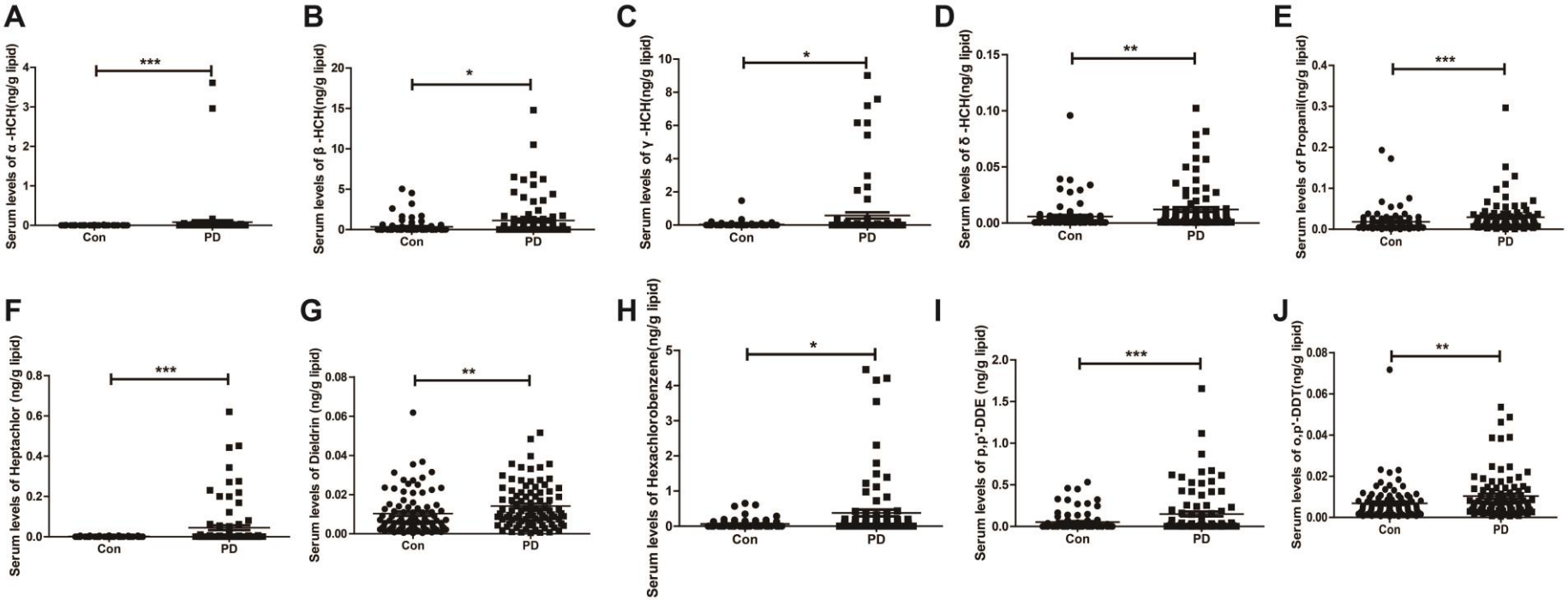

Fig. e-1. Serum levels of pesticides in PD patients and controls (ng/g lipid). (A, B, C, D) Levels of α-HCH (A), β-HCH (B), γ-HCH (C), δ-HCH (D) and were significantly higher in patients with PD vs control participants; (E, F, G, H) Levels of propanil (E), heptachlor (F), dieldrin (G) and hexachlorobenzene (H) were higher in patients with PD than controls; (I, J) Serum levels of p,p'-DDE (I) and o,p'-DDT (J) were elevated in PD patients compared with controls.

α-HCH, α-hexachlorocyclohexane; β-HCH, β-hexachlorocyclohexane; γ-HCH, γ-hexachlorocyclohexane; δ-HCH, δ-hexachlorocyclohexane; p,p'-DDE, p,p'-dichloro-diphenyldichloroethylene; o,p'-DDT, o,p'-dichloro-diphenyl-trichloroethane.

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs. control.

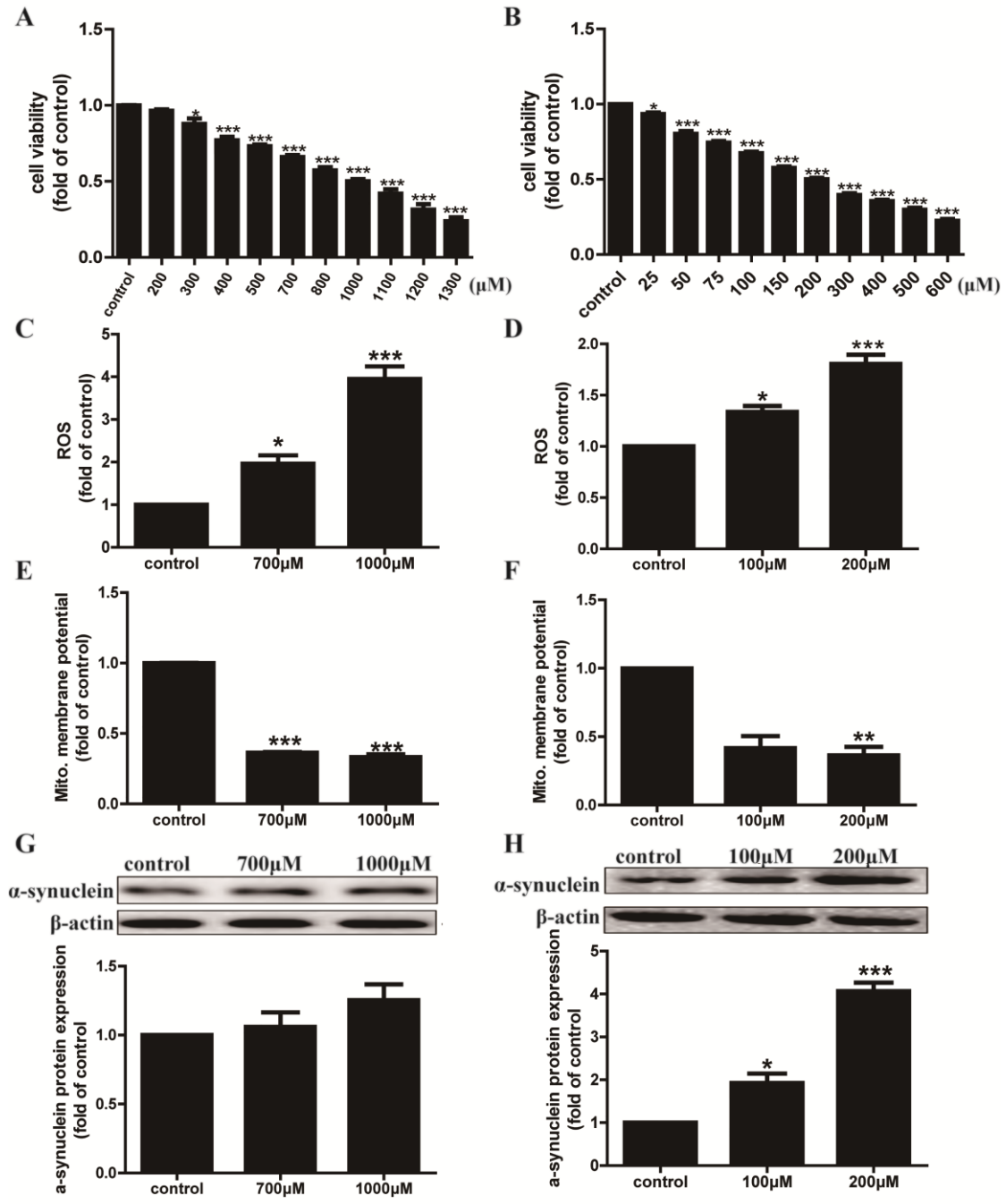

Fig. e-2. The effect of α-HCH and propanil on oxidative injury and α-synuclein aggregation in neuronal cells. (A, B) Cell viability of neuronal cells was decreased upon α-HCH (A) and propanil (B) stimulation for 48h detected by CCK-8. (C, D) ROS production was induced by α-HCH (C) and propanil (D) detected by CM-H2DCFDA. (E, F) The mitochondrial membrane potential was decreased by α-HCH (E) and propanil (F) stimulation detected by JC-10 fluorescent probe. (G) α-HCH had no

significant effect on α-synuclein. (H) α-synuclein aggregation was enhanced by prapanil stimulation.

α-HCH, α-hexachlorocyclohexane. Mito., mitochondrial.

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs. control.