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EDITED BY

Anshu Agrawal,
University of California, Irvine,
United States

REVIEWED BY

Florencia Maria Barbé-Tuana,
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Grande do Sul, Brazil
Irene Martinez De Toda,
Complutense University of Madrid,
Spain
Munis Dundar,
Erciyes University, Turkey

*CORRESPONDENCE

Chun Wang
chun_wang@njmu.edu.cn
Qigang Zhou
qigangzhou@njmu.edu.cn

†These authors share first authorship

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Panic disorder aging characteristics: The role of telomerase reverse transcriptase gene and brain function

Huachen Ding^{1,2†}, Yuan Zhong^{3†}, Na Liu^{1,4}, Huiqin Wu^{1,2},
Huazhen Xu^{1,2}, Yun Wu^{1,2,3,4}, Gang Liu^{1,2,4}, Shiting Yuan^{1,2},
Qigang Zhou^{5*} and Chun Wang^{1,2,3,4*}

¹Nanjing Brain Hospital Affiliated to Nanjing Medical University, Nanjing, China, ²Functional Brain Imaging Institute of Nanjing Medical University, Nanjing, China, ³School of Psychology, Nanjing Normal University, Nanjing, China, ⁴Cognitive Behavioral Therapy Institute of Nanjing Medical University, Nanjing, China, ⁵Department of Clinical Pharmacology, School of Pharmacy, Nanjing Medical University, Nanjing, China

Panic disorder (PD) causes serious functional damage and disability and accelerates the process of individual aging. The pathological basis of PD is the same as that of age-related diseases, which is proposed as a new viewpoint in recent years. Memory decline and social functional impairment are common manifestations of accelerated aging in PD. The function of telomerase reverse transcriptase (TERT) and telomere length (TL) is abnormal in patients with aging and PD. However, the molecular mechanism behind remains unclear. The purpose of this study was to explore the relationship between TERT gene expression (including DNA methylation) and the changes in PD aging characteristics (memory and social function). By TERT gene knockout mice, we found that loss of TERT attenuated the acquisition of recent fear memory during contextual fear conditioning. This study reported that a significantly lower methylation level of human TERT (hTERT) gene was detected in PD patients compared with healthy control and particularly decreased CpG methylation in the promoter region of hTERT was associated with the clinical characteristics in PD. Regional homogeneity (ReHo) analysis showed that the methylation of hTERT (cg1295648) influenced social function of PD patients through moderating the function of the left postcentral gyrus (PCG). This indicates that the hTERT gene may play an important role in the pathological basis of PD aging and may become a biological marker for evaluating PD aging. These findings provide multidimensional evidence for the underlying genetic and pathological mechanisms of PD.

KEYWORDS

telomerase reverse transcriptase gene, DNA methylation, aging, panic disorder, regional homogeneity

Introduction

Panic disorder (PD) impairs social function and memory in patients and is at risk for accelerated aging (Perna et al., 2016). Memory decline and social functional impairment are common manifestations of accelerated aging in PD (Markowitz et al., 1989; Yochim et al., 2013). It is commonly co-morbid with age-related diseases including depression (Kessler et al., 2006) and Alzheimer's disease (Verhoeven et al., 2015). PD and other age-related diseases may have a common pathophysiological basis, which is proposed as a new viewpoint in recent years. The genetic mechanism in PD aging is unclear. Telomere length (TL) is closely related to human aging, especially in memory decline and social function impairment (Verhoeven et al., 2015). The expression of the telomerase reverse transcriptase (TERT) gene plays an important role in regulating TL. The lower expression of the TERT gene generally causes the shorter TL and the hypomethylation or demethylation of the human TERT (hTERT) gene usually reduces hTERT gene expression (Guilleret and Benhattar, 2003). Telomere erosion-mediated cellular senescence is widely used in the study of mental disorders (Lindqvist et al., 2015). Shorter TL has been reported to be relevant to the severity of PD. TERT gene mutation and TERT-knockout can cause stem cell dysfunction, leading to premature aging (Perna et al., 2016). The hTERT gene expression plays an important role in neuronal development (Klapper et al., 2001) and emotional processing, particularly fear processing, fear regulation, and anxiety (Meyer-Lindenberg and Weinberger, 2006). The associations between hTERT gene, depression, and age-related diseases has been confirmed by several studies (Gonzalez-Giraldo et al., 2016; Michalek et al., 2017). The correlations between TERT gene function and anxiety syndrome have been reported in both humans and animals (Teyssier et al., 2012; Kim et al., 2017). Overexpression of the TERT gene is associated with reduced anxiety-like behaviors in male mice, affecting male mice's function in terms of social preference and social competence (Kim et al., 2017). The positive correlation between hTERT gene expression and anxiety level is reported in depressed patients (Teyssier et al., 2012). These findings suggest that the expression of hTERT gene may be involved in the pathology of PD aging.

DNA methylation has been shown to crucially modify gene expression and is an epigenetic process involved in development and aging and sensitive to environmental influences. The differential contribution of DNA methylation in those at risk for PD and has been supported by twins (Alisch et al., 2017) and epidemiologic studies (Bartlett et al., 2017). The hTERT methylation is mostly studied in cancer research (de Wilde et al., 2010) but one study reported the hTERT methylation frequency in Alzheimer's disease (AD) differed from elderly controls (Silva et al., 2008). Since

the genetic effects of the disease may occur by affecting the brain, moreover changes in brain function are more pronounced than changes in brain structure in patients with PD, the investigation of brain function changes associated with the genetic risk for panic disorder is one of an important strategy to find the hTERT gene methylation role. To date, such investigations have mainly focused on the associations of some risk genes with neuroimaging measures or a probed for an "imaging intermediate phenotype." In PD, the study of risk genes is involved in many neurotransmitters and extends to telomerase and other systems gradually (Iurato et al., 2017; Emeny et al., 2018). The amygdala and hippocampus have been commonly reported as neural intermediate phenotypes related to the functional catechol-O-methyltransferase (COMT) gene and so on (Inoue et al., 2015; Sobanski and Wagner, 2017). However, the imaging intermediate phenotype associated with hTERT gene methylation is unclear in PD. Growing evidence suggests that TL partially predicts variations in the brain and aging (Ain et al., 2018; Powell et al., 2019) and the associations between TL and brain function of the amygdala, hippocampus, cuneus, mPFC, and PCG are reported (King et al., 2014; Kim et al., 2016; Powell et al., 2019; Zeng et al., 2019). The effect of TERT gene expression on brain function has been reported in animals (Lee et al., 2010). However, the imaging intermediate phenotype associated with hTERT gene methylation is unclear in PD.

Based on the above-mentioned evidence, we hypothesized that hTERT methylation: modulated brain functional alterations, may influence the amygdala/hippocampus/postcentral gyrus (shared alterations in regions implicated in aging) to increase the possibility of PD aging. The purpose of this study was to explore the relationship between TERT gene expression (including DNA methylation) and the changes in PD aging characteristics (memory and social function). Further, enrich the genetic imaging mechanism of PD aging. To provide biological basis for early prevention, early detection and early intervention to delay the aging process of PD patients.

Materials and methods

Study approval and human subjects

The original sample consisted of 32 eligible PD patients and 22 eligible Healthy controls. They were recruited from Nanjing brain hospital affiliated with Nanjing Medical University through outpatient and public advertising. The selection criteria for PD subjects were as follows: (1) 18–55 years old. (2) Right handedness. (3) Ability to cooperate with all tests and complete them. (4) No other illnesses, no psychotherapy or medical treatment in the past 6 months. We

used mice for a contextual recent fear memory acquisition test (Figure 1A). Four trained psychiatrists used the Mini-international Neuropsychiatric Interview (MINI) to screen all PD patients according to DSM-IV criteria. The scores of the age-match healthy controls on the Hamilton Anxiety Rating Scales (HAMA) should < 7 .

The study was approved by the Ethics Committee of the Nanjing Brain Hospital, affiliates of Nanjing Medical University. All subjects obtained informed consent. The HAMA scale was used to assess the level of anxiety in all subjects. The Panic Disorder Severity Scale (PDSS) was used to assess the severity of panic symptoms in PD patients.

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. $TERT^{-/-}$ mice were backcrossed to FVB/N mice to produce mice heterozygous for $TERT$. The first generation of $TERT \pm$ mice (F1) was used for the production of $TERT^{-/-}$ mice (F2). All experimental subjects were from the fourth or fifth generation after hybridization of $TERT^{-/-}$ mice (F2). Two to three-month-old male mice were used for behavioral tests. In total, there were 15 $TERT^{-/-}$ mice and 21 normal $TERT$ gene mice for testing. Compared with WT mice, the telomerase catalytic activity was not detected in the hippocampus of $TERT^{-/-}$ mice.

DNA methylation data

Peripheral venous blood was drawn from patients by certified nurses at Nanjing Brain Hospital, affiliates of Nanjing Medical University. DNA was extracted from whole peripheral blood (stored in EDTA tubes) with standardized salting out methods and quantified by NanoDrop 2000 (NanoDrop technologies, Wilmington, DE, United States). Genomic DNA (1 μ g; concentration, 20 $\text{ng}/\mu\text{L}$) was bisulfite converted using the Zymo EZ DNA-methylation kit (ZYMO, CA, United States). The multiple-PCR was performed to amplify the bisulfite-modified DNA sequence using indexed primers. The detection of hTERT methylation was performed on Illumina Hiseq (Illumina, CA, United States) using bidirectional sequencing verification with 2×150 bp sequencing mode according to the manufacturer's protocol and to analyze the results. The methylation levels of the hTERT gene promoter were analyzed by MethylTargetTM (Genesky Biotechnologies Inc., Shanghai, China). CpG islands located in the promoter of the hTERT gene were selected from 2 k upstream of transcriptional start site (TSS) to 1 k downstream of the first exon according to the following criteria: (1) 200 bp minimum length; (2) the ratio of

observed/expected dinucleotides CpG > 0.60 ; (3) the content of GC should be no less than 50%. Finally, we selected 7 CpG regions of the hTERT gene promoter including 197 CpG sites (Supplementary Table 1).

Regional homogeneity analyses

The ReHo map of each subject was produced by calculating the Kendall consistency coefficient (KCC) of the ranked time series of a given voxel and its nearest 26 neighboring voxels. The intracranial voxels were extracted to make a whole-brain mask without the non-brain tissue. For standardization purposes, each ReHo map was divided by its own global mean KCC within the whole-brain mask. The generated ReHo maps were spatially smoothed with a $4 \times 4 \times 4$ mm FWHM Gaussian kernel.

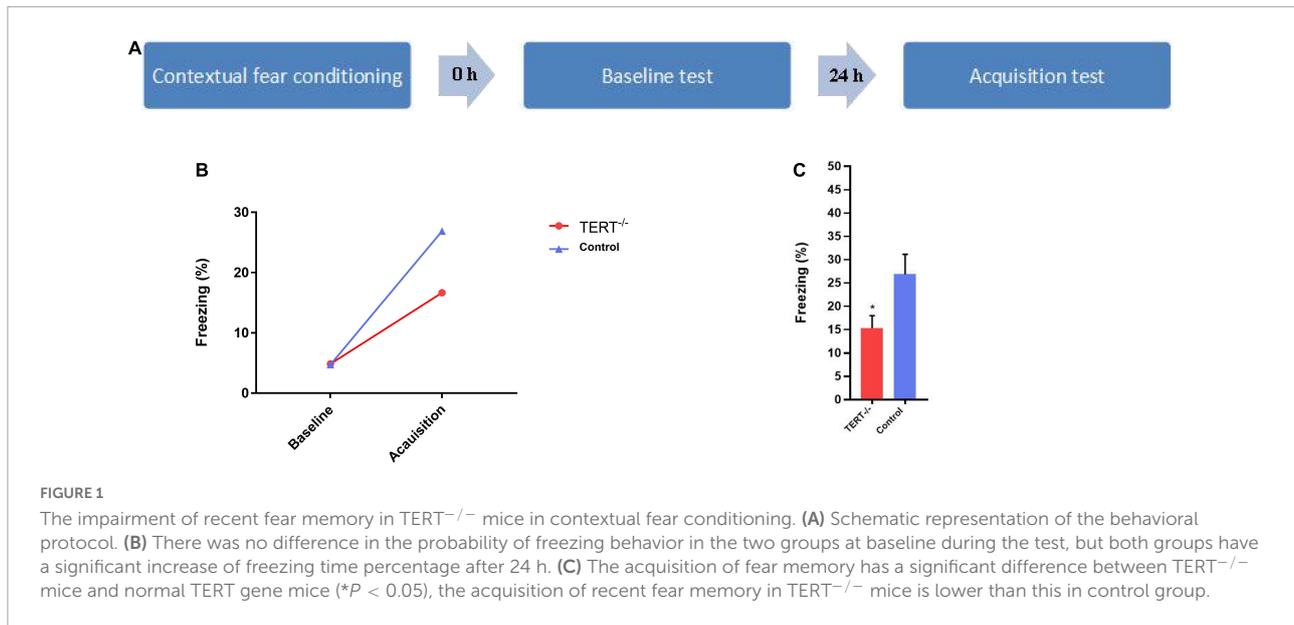
Behavioral tests

We used mice for a contextual recent fear memory acquisition test. Mice were habituated in a conditioning chamber (18 cm \times 18 cm \times 30 cm) which had a grid floor that could be energized and connected to a shock generator. Habituation lasted for 3 min, and percentage of freezing time during those 3 min served as the baseline (freezing was defined as no movement detected for 2 s). After habituation, a foot shock (unconditioned stimulation, 2 s, 0.75 mA) was delivered to the mice. After the shocks, mice remained in the chamber for 1 min and were then returned to their cage. 24 h later, the mice were put back in the chamber, and given the same foot shock for 3 min to obtain the fear acquisition value. During the training session, the mice's behavior was captured by a CCD-camera and the freezing behavior was analyzed by the freezing behavior analyzing software (FreezeScan version 2.00, Clever Sys Inc.).

Statistical analysis

Data analysis was carried out using SPSS software. Differences in dimensional sample characteristics were tested through independent samples *t*-tests; a Two-sample *T*-test was used to compare methylation levels of the hTERT gene between PD patients and healthy controls. Differences in categorical variables were tested using Chi-square tests. Spearman correlations were adopted for bivariate correlation analyses. All data met the normality assumption. We used the GraphPad Prism version 7 to draw the graphs.

To examine the effects of ReHo and hTERT promoter methylation in PD, multiple regression analysis in SPM12 was performed with PD symptoms, methylation, and imaging data. Then, we used REST 1.8 to find the overlap of regions in relation



to methylation and PDSS scores. A linear regression model was performed to discuss the interaction effects, corrected for age and gender.

Results

The deletion of telomerase reverse transcriptase gene attenuated the acquisition of recent fear memory during contextual fear conditioning

Figure 1B shows that there was no difference between $TERT^{-/-}$ mice and wildtype mice in the baseline test. Twenty-four hours after the baseline test, mice were exposed to the same fear chamber to evoke fear memory, and the percentage of freezing time was significantly higher than in the first baseline period for both $TERT^{-/-}$ and wildtype mice. However, the fear acquisition was significantly inhibited in $TERT^{-/-}$ mice compared with wild-type mice (**Figure 1C**).

Demographic and clinical characteristics

The demographic and clinical features of the 32 PD patients and 22 healthy controls at the first stage are listed in **Table 1**. There were no statistically significant differences in age ($t = 0.73$, $p = 0.94$), gender ($\chi^2 = 0.11$, $p = 0.74$), or education level ($t = 1.7$, $p = 0.09$) between PD patients and healthy controls. There were significant differences in HAMA-14 scores ($t = -11.8$, $p = 0.00$) between PD and HC.

Lower methylation in the promoter of human telomerase reverse transcriptase gene in panic disorder associated with high severity of panic disorder

The methylation of hTERT gene differed significantly between PD patients and healthy controls (**Figure 2**), with PD patients exhibiting decreased average methylation ($p = 0.0071$). The CpG7 Island of the hTERT gene promoter was hypomethylated in PD patients compared to healthy controls ($p = 0.0133$). There were 23 CpG sites in CpG7 region and of them, 13 (cg5-17) differential CpG sites were significantly hypomethylated. In addition, hypomethylation was detected in 4 CpG sites in other islands in the hTERT gene promoter in PD patients. These 17 CpG sites were analysis for correlations with clinical characteristics.

The significant results are shown in **Figure 2**, located inside CpG7, the methylation level of 3 CpG sites located inside CpG7 was significantly negatively associated with monthly income and 1 CpG site was significantly positively associated with the level of realistic physical exercise (**Figure 3**). No significant correlations were found between methylation level and gender, course of the disease, age, the age of onset.

The Pearson correlation analysis showed the negative relationship between the methylation alterations of the hTERT gene and the severity of PD, include total PDSS, PDSS6, PDSS7, and SFI scores (social function impairment scales) (**Figure 3**). There were significant inverse relations for the methylation mean and the scores of all four PDSS scales. The methylation level of 2 CpG sites (cg6 and cg17) located in the CpG7 region were negatively relevant to the scores of all the PDSS scales.

TABLE 1 Demographic and clinical characteristics of total samples.

	PD (SD)	HC (SD)	Different	
Subjects (<i>n</i>)	32	22		
Male/Female	16/16	12/10	0.11 ^a	0.74
Age (year)	33.1 (7.4)	33.3 (7.2)	0.73 ^b	0.94
HAMA-14	20.3 (7.0)	2.2 (1.9)	-11.8 ^b	0.00
Education (years)	13.9 (3.3)	15.6 (3.8)	1.7 ^b	0.09

a = χ^2 ; b = independent sample *t*-test. PD, panic disorder; HC, healthy control; SD, standard deviation; HAMA-14, the total scores of Hamilton Anxiety Scale 14 Items.

Hypomethylation of the human telomerase reverse transcriptase gene promoter and regional homogeneity analysis

In the PD group, there were positive correlations between ReHo and methylation levels at hTERT average (Table 2 and Figure 4).

Correlations between social function impairment, panic symptom severity and human telomerase reverse transcriptase-modulated differences in regional homogeneity

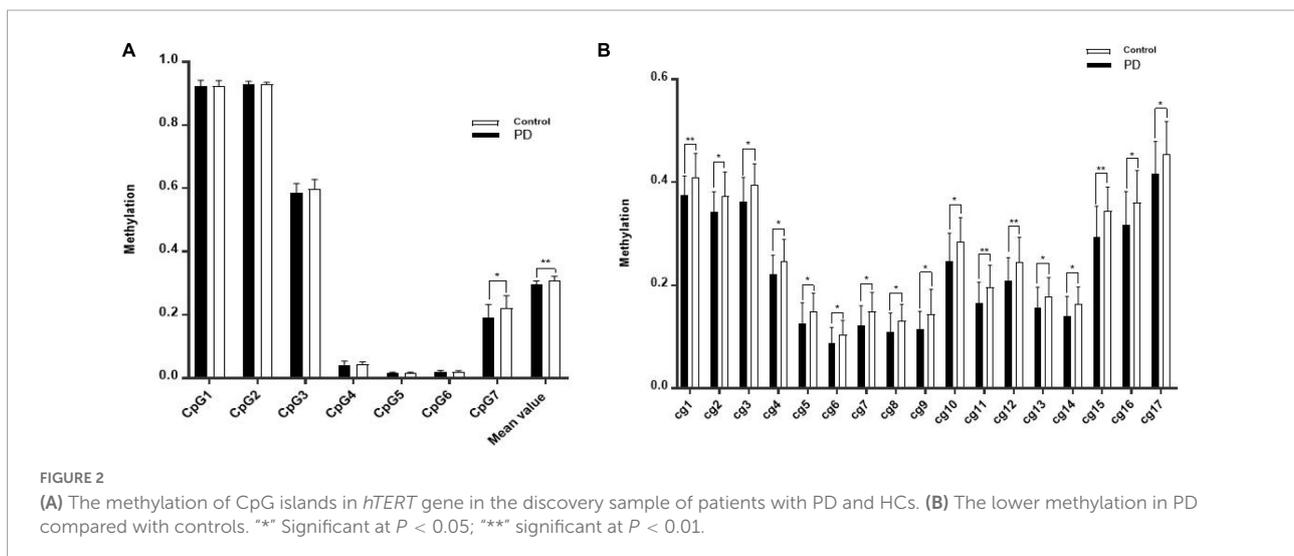
No direct association between ReHo and the total PDSS score was found, however, there was a negative correlation between SFI score and ReHo in the left PCG and IPL. The aberrant ReHo in left PCG and IPL was linked to both hTERT methylation and SFI in PD patients. Average ReHo values were hence extracted from the overlapping region of the two

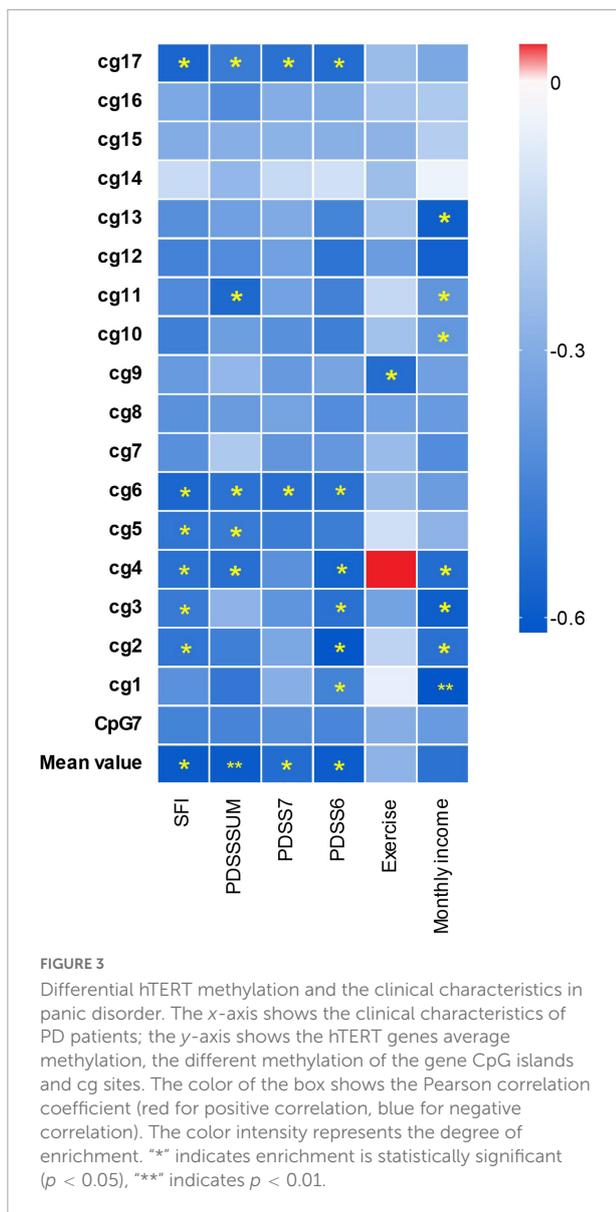
clusters showing significant effects of hTERT methylation and SFI in PD group; 2 significant overlapping clusters, b/b1, were found (Table 3 and Figure 4). Within these clusters, negative correlations between social function damage and hTERT gene average/cg6 (cg1295648) methylation were observed (hTERT average: $r = -0.601$, $p = 0.011$; cg6: $r = -0.557$, $p = 0.02$). The results also showed a significant negative correlation between ReHo of b1/b and hTERT gene average/cg6 (cg1295648) methylation (hTERT average: $r = -0.757$, $p < 0.0001$; cg6: $r = -0.736$, $p = 0.001$). Because the mean value of hTERT methylation could not be accurately quantified, the cg6 was chosen as an independent variable to study the interaction between brain function and methylation. To better understand the role of PCG and IPL, the values of the b1 cluster were divided into the values of b2 (left PCG)/b3 (left IPL) clusters (Figure 4). ReHo in left PCG (b2) and IPL (b3) was related to severity of PD (PCG: $r = -0.609$, $p = 0.009$; IPL: $r = -0.569$, $p = 0.017$).

After controlling for age and sex, the linear regression models revealed an interaction between hypomethylation of cg6 and ReHo modulated by the CpG site in the left PCG (b2) ($p = 0.043$, Table 4). In PD, the hypomethylation of the CpG site decreased social function by moderating the function of left PCG. The moderating effect equation is as follows: $Y = 132.715 \times 1 X - 8.478 \times 1 X$ ($R^2 = 0.634$, $X_1 =$ cg6 methylation level, $X =$ the value of b2 clusters) (Figures 4A,B).

Discussion

The ability of functional brain imaging techniques to explore genes is well-recognized. Recent PD studies have used functional imaging to examine the effect of a panic disorder-related brain-based intermediate phenotype on





neural activity (Gechter et al., 2019; Gottschalk et al., 2019). But few studies have shown the neural intermediate phenotype of anxiety in relation to methylation (Oh et al., 2013; Ziegler et al., 2015). This study is the first to demonstrate that ReHo in specific brain regions is affected by hTERT promoter methylation (especially, cg1295648) in PD. This study reported significant hypomethylation of the hTERT promoter in panic disorder patients as well as significantly lower methylation of CpG sites in the CpG7 promoter region. The methylation level showed significant correlations with disease symptoms (severity of PD, especially the degree of social function damage), sociological data related to stress (e.g., per-capita income) (Powell-Wiley et al., 2020) and the engagement of protective behaviors (e.g., exercise) (Nomikos et al., 2018). The association

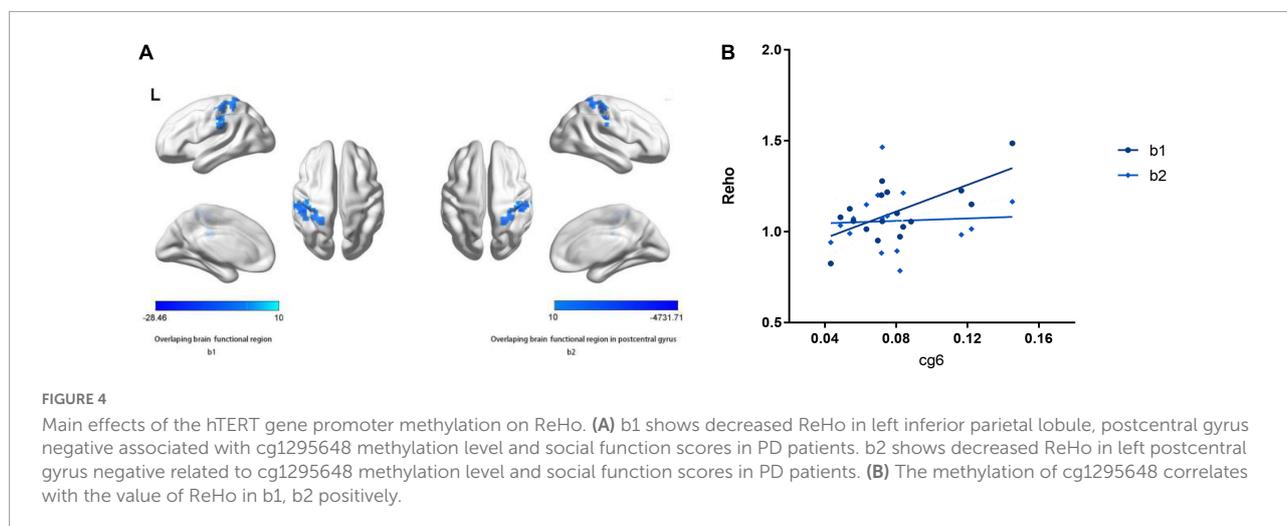
between higher frequency of exercise/higher income and increased methylation is consistent with other studies that suggested exercise can delay telomere shortening and lower-income can accelerate telomere shortening (Nomikos et al., 2018; Powell-Wiley et al., 2020). These results are corroborated by previous findings that TL may be shorter in PD patients than in healthy controls (Lindqvist et al., 2015).

Genes affect the changes in brain structure and function, which are thought to be mediated by epigenetics. DNA methylation is a widely studied epigenetic modification. 60 reliable studies involving the relationship between DNA methylation and human brain structure/function are reviewed (Wheater et al., 2020). These studies report DNA methylation-MRI associations for psychiatric disorders, including schizophrenia, major depression, post-traumatic stress disorder, social phobia, and so on. All studies used alternative tissues to detect methylation, and 46 of them explored the relationship between DNA methylation and brain structure and function by collecting blood samples. Combining genetic and fMRI data, this study observed that the TERT gene may play an important role in memory and functional impairment associated with brain aging and is involved in the pathological mechanisms of PD. It has been inferred that the gene-brain-behavior relationship can be largely attributed to the TERT gene, and its methylation alterations may lead to symptoms (e.g., memory and functional impairment) related to aging. This is the first study to demonstrate that decreased ReHo in left PCG and IPL was associated with the TERT gene in patients with PD. At the same time, social function impairment scores, represented by the PDSS scale, displayed a negative association with ReHo in left PCG and IPL in PD patients. The SFI of PD patients has a relationship with decreased ReHo in left PCG which is regulated by the hTERT gene methylation. Previous studies have shown that neurobiological alterations of inferior parietal lobule (Magnotta et al., 2014; Lai and Wu, 2016) and postcentral gyrus (Maggioni et al., 2019) are implicated in the pathophysiology of PD, and some of the negative alterations they reported were consistent with our research (Lai and Wu, 2016). Considering the IPL is important for the mental representation of social space (Husain and Nachev, 2007), the IPL abnormality in PD makes it difficult for people to respond to societal stimulation and is negatively related to the severity of social avoidance (Irle et al., 2014; Cardillo et al., 2018). The PCG has also been linked to social avoidance, with two studies reporting that PCG abnormalities were related to stronger social avoidance (Syal et al., 2012). Decreased activity of the PCG in response to sad faces has been observed in people with high attachment avoidance (Li et al., 2014). In healthy people, the PCG and the IPL are thought to play putative roles in avoidance behaviors and responses to the immediate intention of other persons (Conty et al., 2012). It means these functional changes in IPL and PCG may play a key role in social function related to

TABLE 2 Significant clusters show the effects of PD and hTERT methylation level on regional homogeneity.

	MNI coordinates			Cluster voxels	T-value	Area	Hemisphere
	x	y	z				
hTERT average	-42	-48	39	133	9.4383	PCG	L
				129		IPL	
cg6	-54	-24	33	191	5.8263	PCG	L
				109		IPL	
SFI	-54	-24	27	114	-8.2444	PCG	L
				87		IPL	
				67		Thalamus	

hTERT ave: mean value of hTERT promoter methylation. SFI: significant clusters on ReHo association with the social function impairment of PD patients.



PD, and the moderating model in our study supports this view. Overexpression of TERT was associated with reduced social interaction and decreased preference for novel social interaction (Rhee et al., 2018). Telomere shortening has been observed in autistic children with social interaction disorders (Li et al., 2014).

TABLE 3 Significant overlapping clusters that associated with hTERT gene methylation and the degree of social functional impairment in PD.

Methylation	MNI coordinates			Cluster voxels	Region
	x	y	z		
hTERT ave(b)	-45	-33	45	65	Postcentral_L
				49	Parietal_Inf_L
cg6(b1)	-54	-24	30	67	Postcentral_L
				46	Parietal_Inf_L
cg6(b2)	-54	-24	39	56	Postcentral_L

hTERT ave: mean value of hTERT promoter methylation. b1: the significant overlapping clusters that associated with the methylation of cg1295648 and the degree of harm of patients' social function. b2: the significant overlapping clusters located left postcentral gyrus, that associated with the methylation of cg1295648 and the degree of harm of patients' social function.

TL in the parietal cortex has a potential association with its brain function (King et al., 2014; Cardillo et al., 2018). These studies built on the findings of the studies discussed above to suggest that the changes in TERT expression could indirectly affect the social function of individuals with PD. These hTERT promoter methylation-modulated brain functional alterations in ReHo were related to SFI in PD patients. PCG is the main region affected by hTERT gene methylation and has a major impact on social functioning in the PD group, possibly owing to the unique role of PCG in abnormal perception and sensory processing, and is involved in fear processing and the production of fear memory (Shang et al., 2019). PCG is also abnormal when comparing PD with GAD and MDD (Maggioni et al., 2019). Changes in PCG function are commonly reported in memory impairment and aging-related diseases (Kropf et al., 2019; Zeng et al., 2019). Memory is also one of the indicators for assessing aging. Anxiety and reduced social interaction can be partly attributed to memory impairment (Veluri, 2019). Memory decline will inevitably affect social communication and social behavior (Petkus et al., 2017), and one study confirms that spatial working memory is related to social factors scores (Fleck et al., 2019). Our findings indicate that disrupting the expression

TABLE 4 Linear regression models of brain function and interaction effect with cg1295648 CpG methylation in social function damage in PD.

		B	t	P	R ²	R ² change	Sig. F change
Model 1	cg6(a)	-8.968	-0.517	0.613	0.551	0.551	0.004**
	overlapping region(b1)	-8.571	-2.737	0.016*			
Model 2	A	-23.124	-1.369	0.194	0.666	0.115	0.054
	b1	-9.301	-3.295	0.006*			
	c1	123.272	2.117	0.054			
Model 3	a	-13.614	-0.746	0.468	0.493	0.493	0.009**
	PCG_L_overlap(b2)	-7.068	-2.241	0.042*			
Model 4	A	-26.98	-1.572	0.14	0.634	0.141	0.043*
	b2	-8.478	-2.973	0.011*			
	c2	132.715	2.239	0.043*			
Model 5	a	-14.406	-0.862	0.403	0.531		0.005**
	IPL_L_overlap(b3)	-6.939	-2.563	0.023*			
Model 6	a	-21.95	-1.144	0.273	0.554		0.422
	b3	-6.255	-2.187	0.048*			
	c3	53.131	0.829	0.422			

Linear regression models for the association of hTERT promoter methylation-modulated alterations in ReHo (b1, b2, b3), social function damage and *M*-value cg1295648 methylation are shown for a model adjusted for sex, age, monthly income. Model 2/4/6 included interaction terms (c1/c2/c3) for cg1295648 CpG methylation and mention value of ReHo in b1/b2 located in left postcentral gyrus/b3 brain region located in left inferior parietal lobule. * $p < 0.05$, ** $p < 0.01$.

of TERT in mice can influence the acquisition of recent fear memory. We can boldly speculate that the pathological changes of TERT gene may affect memory acquisition and lead to social function impairment. TERT^{-/-} mice had terrible spatial memory and the TERT gene had the ability to regulate the formation of spatial memory by adjusting the number and morphological complexity of neurons (Zhou et al., 2017). TERT knockout mice generally have shorter telomeres which are typically associated with poor memory include episodic and spatial (Czepielewski et al., 2018; Powell et al., 2018), and lead to episodic memory-related learning activities defects (Valdes et al., 2010). As a result, knockout mice may have a weaker perceptive ability in the contextual conditioning chamber or impaired memory, which may lead to a reduced ability to acquire recent fear memory as quickly as the control group. Some studies have reported that poor spatial memory in mental patients is associated with reduced activation (Eckfeld et al., 2017) and reduced resting-state functional connectivity of PCG and IPL (Ren et al., 2019). Further investigation is needed to confirm the relationship between IPL/PCG, TERT, and fear memory and between memory mediated by the TERT gene and social function to refine the gene-related pathophysiological mechanism in PD aging.

Finally, some limitations to the present study should be mentioned. First, the sample size of our study was not large enough. Second, whether the methylation of hTERT in peripheral blood can directly affect brain function and whether it is consistent with gene methylation in the brain is unclear. Third, the evaluation of social function needs to be improved. In future studies, it might be helpful to include SAD as a control group to determine

whether the gene methylation has a wide influence on social function.

In conclusion, our results suggest that the hTERT gene may play an important role in the pathogeny of PD aging. Memory impairment and social dysfunction are both signs of aging. This study's findings provide evidence that the deletion of the hTERT gene attenuates the acquisition of fear memory, and hTERT methylation modulates the local brain function of postcentral gyrus to affect the social function of PD patients. These findings could potentially help us develop novel ways to predict and evaluate the aging risk of panic disorder and enrich the research field of telomere genetic imaging in PD.

Data availability statement

The original contributions presented in this study are publicly available. This data can be found here: <https://www.ncbi.nlm.nih.gov/>, accession: PRJNA793355. The data and code that support the findings of the present study are available from the corresponding author through reasonable request. The data and code sharing adopted by the authors comply with the requirements of the funding institute and with institutional ethics approval (ClinicalTrials.gov, ID: NCT03199625, URL: <https://clinicaltrials.gov/>).

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Nanjing

Brain Hospital, affiliates of Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the Cleveland Clinic and Nanjing Medical University.

Author contributions

CW and QZ designed the study and supervised the conduct of the study. GL, HW, and SY contributed to the data collection. HX, YW, and YZ provided methodological advice. HD and HX performed the data analysis and results interpretation. HD, CW, and QZ drafted the manuscript. All authors reviewed and approved for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnagi.2022.835963/full#supplementary-material>

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