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Age-specific associations among functional *COMT* Val^{158}Met polymorphism, resting parasympathetic nervous control and generalized anxiety disorder

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ABSTRACT

The functional Val^{158}Met polymorphism (*rs*4680) of the Catechol-O-Methyltransferase (*COMT*) gene has been implicated in generalized anxiety disorder (GAD); however, the underlying neural mechanisms remain unexamined. Recent evidence reveals that low resting parasympathetic (vagal) control is an endophenotypic predictor of anxiety, while the effect of *COMT rs*4680 differs at different ages. Thus, we examined whether the *COMT* Val^{158}Met variant could increase the risk of GAD through decreased resting parasympathetic nervous control in an age-specific manner. *COMT rs*4680 polymorphism was genotyped in 1,655 Han Chinese adults (1,142 healthy subjects and 513 patients with GAD; age: 20–65). High-frequency power (HF) of heart rate variability (HRV) was used to measure resting state parasympathetic nervous regulation. Non-genetic factors, such as gender, smoking status, medication use and comorbidity conditions, were treated as covariates. After adjusting for relevant covariates, there was a significant age x *COMT* genotype interaction on resting HF of HRV. In younger adults, Met allele carriers had a significantly lower HF index; however, older adults exhibited the opposite pattern, with Val/Val homozygotes exhibiting decreased HF values. Moreover, reduced HF-HRV is associated with increased risk of GAD. Finally, pathway analysis revealed a significant indirect effect of *COMT* on the risk of GAD via reduced resting HF-HRV, in the aforementioned age-dependent manner. Our findings are the first to demonstrate that *COMT* Val^{158}Met polymorphism is associated with risk of GAD via reduced resting parasympathetic nervous control, an age-specific risk pathway.

1. Introduction

Generalized anxiety disorder (GAD) is characterized by excessive, long-lasting worry associated with restlessness, fatigue, poor concentration, irritability, muscle tension, and insomnia. In addition, it is further characterized by a number of autonomic nervous system (ANS) symptoms, including hot flashes, palpitations, sweating, and shaking (Jetty et al., 2001; Tully et al., 2013). GAD is highly prevalent and debilitating, with a lifetime prevalence in the community estimated to range from 5.1%–11.9% (Kessler et al., 2008; Wittchen et al., 1994). Twin and family studies have suggested that genetic factors are important in the etiology of GAD, estimating 32% of the variation in the risk of GAD being attributable to genetic variability (Hettema et al., 2001). As such, interest has been focused on the identification and verification of specific genes that contribute to the development of GAD.

One of the most promising candidate genes for GAD is *COMT* (chromosome 22q11), encoding the eponymous enzyme Catechol-O-methyltransferase (COMT). COMT induces the transfer of a methyl group from S-adenosylmethionine to catalyze catecholamines (e.g., dopamine and norepinephrine), which are essential neurotransmitters in the prefrontal cortex (Clark and Noudoost, 2014), a known brain region involved in anxiety (Kim and Whalen, 2009). A common single nucleotide polymorphism (SNP) *rs*4680 (G > A) in the *COMT* gene, resulting in a valine (Val) to methionine (Met) substitution at protein position 158 (Val^{158}Met), alters COMT expression, with the Val allele

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presenting higher COMT activity than the Met allele (Lachman et al., 1996). Notably, the high-activity *COMT* Val allele has been linked with increased risk of anticipatory worry (the core feature of GAD), persisting generalized anxiety and GAD (Hettema et al., 2008; Mc Fie et al., 2017; Olsson et al., 2007). Conversely, research has also revealed that low-activity Met allele of *COMT* is associated with anticipatory worry, anxiety sensitivity and symptomatology related to anxiety (Baumann et al., 2013; Enoch et al., 2003; Olsson et al., 2005). However, the neurobiological reasons for these completely conflicting results are currently unclear.

COMT enzyme activity and protein expression have been shown to increase with age across the human lifespan (Tunbridge et al., 2007). In addition, healthy individuals carrying the *COMT* Val/Val genotype have higher grey matter density in early adulthood, but this difference disappears as age increases (Rowe et al., 2010). Furthermore, Smith and Boettiger (2012) have reported that age modulates the *COMT rs*4680 effect on task performance of delay-discounting, a behavioral tendency to choose immediate over delayed rewards. Moreover, effects of *COMT* Val^{158}Met genotypes have been shown to have opposite influences in adolescents and adults, with Val-allele homozygous adults exhibiting elevated, and adolescents presenting diminished, resting-state functional connectivity of brain regions including the prefrontal cortex, compared to Met-allele homozygotes (Meyer et al., 2016). Thus, age itself may play a role in moderating the effect of *COMT* Val^{158}Met polymorphism on neural function and related behavioral phenotypes.

Growing evidence has shown that ANS dysregulation, indexed by heart rate variability (HRV), plays a critical role in the pathogenesis of anxiety (Minassian et al., 2014, 2015). Indeed, we have previously reported that patients with GAD are associated with reduced resting parasympathetical (vagal) control (Chang et al., 2013). Furthermore, young people who are at risk for anxiety disorders show decreased vagal regulation than a control group in resting conditions (Balle et al., 2013). Moreover, studies have demonstrated that low parasympathetically mediated HRV predicts greater risk for the future develpoment of general anxiety symptoms (Greaves-Lord et al., 2010; Kogan et al., 2012). As resting HRV is highly heritable, with estimated heritability ranging from 32% to 71% (Wang et al., 2005), lower vagal control at rest has been considered an important anxiety endophenotype (Crisan et al., 2009; Thayer and Lane, 2009), a heritable, primarily state-independent biomarker that is associated with related illness (e.g., GAD). Because endophenotypes are more directly related to relevant gene action than to the endpoint disorder (i.e., GAD), an endophenotype-based pathway approach may therefore aid gene-finding strategies (Gatt et al., 2008; Gottesman and Gould, 2003). Importantly, researchers have recently proposed that endophenotype can be manifested only at certain ages when taking into considertion of the importance of developmental and/or epigenetic factors (Flint and Munafo, 2007; Hasler et al., 2006).

In addition, experimental evidence has demonstarted that the neurotransmitter dopamine attenuates prefrontal cortical suppression to the amygdala (Rosenkranz and Grace, 2001), one of the major components of the central autonomic network (Benarroch, 1993). Recently, research has further shown that prefrontal cortex functional conneticity with the amagdala is associated with vagus-mediated HRV (Sakaki et al., 2016). Moreover, prejunctional beta-2 adrenoceptor-mediated reduction in cardiac norepinephrine spillover has also been reported to be related to reflex vagal HR responses (Kubo et al., 2005). Thus, taken all together, given that COMT is responsible for catecholamine metabolism (Clark and Noudoost, 2014), it seems reasonable to assume that GAD may be conferred by the *COMT* Val^{158}Met polymorphism through effects on decreasing resting parasympathetic control, and that such effects are determined in an age-dependent way. To our knowledge, however, no previous study has investigated the potentially age-specific association of the *COMT rs*4680 polymorphism with vagus-mediated HRV to provide a neurophysiological insight into the complex role of *COMT* variation in GAD. ANS functions can also be affected by several factors, including perceived stress level, gender, smoking status, habitual physical exercise, use of medication, and morbidities of physical diseases and/or mental disorders (Chang et al., 2017; Cohen et al., 1999). Therefore, using a highly-controlled manner can minimize the effects of these confounding variables to precisely reveal the possible age-dependent pathways of the *COMT* Val^{158}Met polymorphism, resting vagal control and GAD.

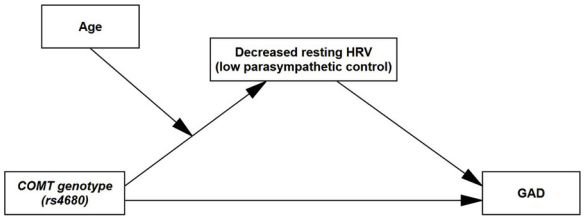

Fig. 1. Theoretical model of the present study.
Abbreviations: *COMT*, Catechol-O-Methyltransferase gene; HRV, heart rate variability; GAD, generalized anxiety disorder.

Using a large cohort of Han Chinese adults and based on a genotype-endophenotype-phenotype pathway model, the current study, adjusting for the relevant confounding factors, aimed to test whether functional *COMT* Val^{158}Met polymorphism has an age-specific correlation with decreased parasympathetically mediated HRV, and if so, whether it is further associated with increased risk of GAD. The hypothetical model is shown in Fig. 1.

2. Materials and methods

2.1. Participants

The Institutional Review Board of the Tri-Service General Hospital (TSGH), Taipei, Taiwan approved the study protocol. We obtained written informed consent from all participants and fully explained the procedures of the study. To reduce the ethnic differences in the allele frequencies of *COMT*, the study participants were recruited from the Han Chinese population in northern Taiwan. All participants were unrelated Han Chinese. In total, we included 1,655 adult civilian subjects for the statistical analysis. Their demographic data and health-related variables were obtained, including age, gender, body mass index (BMI: kg/m^2), smoking status (yes/no) and weekly exercise level (nil; 1–2 times/week; or ≥3 times/week).

Among them, 513 subjects were patients diagnosed with GAD (235 men and 278 women; mean age 44.4 ± 13.5, range 20–65 years), who were recruited from clinical settings at TSGH. Each individual was evaluated by an attending psychiatrist using the Chinese Version of the Mini-International Neuropsychiatric Interview (MINI), which provides standardized assessment of the most common psychiatric diagnoses in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) criteria (Sheehan et al., 1998; Si et al., 2009). All these patients met the DSM-IV-TR criteria for GAD and none of them had a comorbid diagnosis of schizophrenia, bipolar disorder, substance dependence or organic brain disease (e.g., stroke). However, comorbid diagnoses of depressive disorders (i.e., major depression, dysthymia or depressive disorder not otherwise specified) or other anxiety disorders (e.g., panic disorder, obsessive-compulsive disorder, phobic disorders) were not criteria for exclusion. Likewise, all the comorbid psychiatric diagnoses were made using the Chinese Version of the MINI. Furthermore, participants' physical morbidity including hypertension, heart disease (e.g., coronary heart disease, abnormal heart rhythm), diabetes mellitus, dyslipidemia (e.g., hypercholesterolemia, hypertriglyceridemia) and other chronic diseases (e.g., kidney, liver and thyroid diseases) and use of medications (e.g., antidepressants, benzodiazepines, and cardiovascular drugs) were also recorded on the basis of self-report complemented by chart review. This

patient group included 258 GAD patients who were free of any comorbidity and drug use (i.e., drug free for at least two weeks before enrollment).

In addition, our sample also included 1,142 healthy participants (551 males and 591 females; mean age: 38.4 ± 10.3, range 20–65 years). Recruitment and exclusion processes have been described in detail elsewhere (Chang et al., 2017; Huang et al., 2018). Briefly, participants received a medical checkup at TSGH, which consists of physical examination, blood pressure measurement, electrocardiography (ECG), thoracic radiography, and biochemical analyses (e.g., fasting plasma glucose, serum triglyceride, total cholesterol and thyroid function test). Subjects here were free of organic diseases, including cardiovascular disease, kidney or liver disease, metabolic disorders, malignancy, neurological disorder, or obesity (BMI ≥ 30 kg/m^2). They were also free of psychiatric disorders (e.g., affective disorder, anxiety disorder, schizophrenia, and substance use disorders), based on our screening using the Chinese Version of the MINI (Sheehan et al., 1998; Si et al., 2009) by a senior research assistant. Furthermore, none of them were using any medication, as determined by self-report, during the month preceding the beginning of the study.

2.2. Genotyping of the COMT Val^{158}Met polymorphism

Genomic DNA was extracted from venous blood samples using the QIAamp® DNA Mini Kit (Qiagen®, Valencia, CA). The SNP Val^{158}Met of the *COMT* gene (*rs*4680; G472A) was genotyped utilizing a fluorogenic 5'-nuclease TaqMan® allelic discrimination method, as described elsewhere (Chen et al., 2016), on the ABI Prism 7900 Sequence Detection System (Applied Biosystems®, USA). The number of Met/Met, Met/Val and Val/Val genotypes was 94, 659 and 902, respectively. Individuals with at least one Met allele were thus integrated with a group of Met allele carriers (n = 753) and compared with the Val-allele homozygotes (n = 902); this approach has been previously used to address skewed genotypic distributions (Zhang et al., 2015).

2.3. Assessment of resting parasympathetic activity

Participants' resting HRV data were obtained using an SA-3000 P HRV analyzer (Medicore Co., Ltd., South Korea) in a quiet and temperature-controlled room. After 15 min of rest, each subject received a 5-min ECG recording, with normal breathing in a sitting position. The ECG monitoring was performed in the daytime (8:00 a.m.– 4:00 p.m.) to avoid the effect of potential diurnal variations in HRV.

The SA-3000P HRV analyzer was used to identify the beat-to-beat variations in heart rate (Chang et al., 2017). Using a fast Fourier transform-based nonparametric algorithm, the power spectrum was then converted into frequency-domain indices, which consisted of the high-frequency power (HF) (0.15–0.4 Hz) and low-frequency power (LF) (0.04–0.15 Hz) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The HF reflects parasympathetic nervous control and is the most commonly reportedly measure of HRV in the anxiety literature (Chalmers et al., 2014). A natural logarithmic transformation was used to reduce the skewness of the HF-HRV measure.

2.4. Assessment of stress levels

Participants are asked to rate their stress over the previous month with the Chinese version of the Perceived Stress Scale (PSS), a 14-item self-reported questionnaire (Chu and Kao, 2005). The total scores for the PSS were the sums answered on all the items (never = 0, almost always = 4). Higher scores reflect increased levels of perceived stress. The PSS is a well-known and useful tool used to assess the levels of psychological stress in the last month (Cohen et al., 1983).

2.5. Statistical analysis

The Hardy-Weinberg equilibrium was tested for the *COMT* genotype distributions. We used Student's t-test to compare the means of continuous variables, while the chi-square (χ^2) statistic was used to assess relationships between categorical variables within the *COMT* genotype groups. To control the non-genetic confounders, Spearman correlation was used to test the associations between resting HF index and demographic/clinical variables (e.g., gender, comorbidity status, medication use). Those variables correlated with resting HF-HRV were then used as covariates in all analyses. SPSS macro PROCESS (model 1) (Hayes, 2015), was applied to determine whether age moderates the associations between *COMT* genotypes and resting HF-HRV using bias-corrected bootstrapping procedure (10,000 bootstrap samples). The Johnson-Neyman technique further identifies the particular values within the measurement range of the moderator (age), where the conditional effect of *COMT* on resting HF-HRV transitions between not statistically significant to statistically significant. Logistic regression models were used to examine the effect of resting HF-HRV on the risk of GAD. Finally, we tested whether age moderates the indirect (mediation) effect model linking *COMT* genotypes to GAD via resting HF index by using PROCESS model 7 (Hayes, 2015), based on bootstrap (n = 10,000) bias correction method. In this moderated mediation model, covariates were entered in prediction to both the mediator (HF-HRV) and the dependent variable (GAD). Post-hoc analyses were conducted to examine the 95% bootstrapped confidence intervals (95% CI) at the 10th, 25th, 50th, 75th and 90th percentiles of age in our sample. When a 95% CI does not include the value of zero, it can be considered that there is a statistically significant result. The path coefficients between variables were represented with unstandardized regression weights. P values of < 0.05 (2-sided) were considered to be statistically significant.

3. Results

3.1. Sample characteristics based on COMT genotype groups

The genotypic frequency of *COMT* Val^{158}Met polymorphism in our study population did not deviate significantly from the Hardy-Weinberg equilibrium expectations (χ^2 = 3.44, $p > 0.05$). The clinical and demographic characteristics of study participants with different *COMT* genotypes are shown in Table 1. In addition, group comparisons of demographics and clinical variables among the GAD group and healthy control group were also presented in Supplementary Table 1.

3.2. Non-genetic factors correlated with resting HF-HRV

Relationships between HF-HRV and potential confounding variables are summarized in Table 2. Women had significantly decreased resting HF values ($r = -0.103$, $p < 0.001$) compared to men. Current smokers had increased resting HF-HRV (r = 0.048, p = 0.043). Participants with higher BMI had lower resting HF index ($r = -0.073$, p = 0.002). Diagnosis of depressive disorder ($r = -0.090$, $p < 0.001$), other anxiety disorder ($r = -0.052$, p = 0.029), hypertension ($r = -0.073$, p = 0.002), heart disease ($r = -0.090$, $p < 0.001$), diabetes mellitus ($r = -0.065$, p = 0.006), dyslipidemia ($r = -0.066$, p = 0.006), or other chronic diseases ($r = -0.069$, p = 0.004) were all negatively correlated with resting HF index. In addition, the use of medications including antidepressants ($r = -0.104$, $p < 0.001$), benzodiazepine ($r = -0.107$, $p < 0.001$), antipsychotic drugs ($r = -0.093$, $p < 0.001$) and cardiovascular drugs ($r = -0.087$, $p < 0.001$) were also inversely related to resting HF-HRV. Thus, these resting HF index-related variables were entered as covariates in the current analyses.

Table 1
Demographic data and clinical characteristics of the study cohort (n = 1655), stratified by *COMT* Val^{158}Met genotypes.

Characteristics	Met allele carriers (n = 753)	Val/Val homozygotes (n = 902)	χ^2 or t	p
Age, year	40.6 ± 11.8	39.9 ± 11.7	1.17	0.24
Female sex, n (%)	403 (53.5)	466 (51.7)	0.57	0.45
BMI, kg/m^2	22.8 ± 3.26	22.7 ± 3.29	0.82	0.41
Current smoker, n (%)	139 (18.5)	193 (21.4)	2.21	0.14
Weekly regular exercise			0.83	0.66
Nil, n (%)	414 (55.0)	514 (57.0)		
1-2 times/ week, n (%)	205 (27.2)	229 (25.4)		
≥3 times / week, n (%)	134 (17.8)	159 (17.6)		
Mean HR, beats/min	72.4 ± 11.5	72.3 ± 10.6	0.23	0.82
SBP, mmHg	115.9 ± 14.8	115.5 ± 15.8	0.56	0.57
DBP, mmHg	75.5 ± 10.7	75.1 ± 10.8	0.74	0.46
PSS, scores	23.6 ± 9.53	24.1 ± 9.54	1.08	0.28
GAD, n (%)	246 (48.0)	267 (52.0)	1.81	0.18
Depressive disorder, n (%)[a]	43 (5.7)	52 (5.8)	0.002	0.96
Other anxiety disorder, n (%)[b]	23 (3.1)	21 (2.3)	0.84	0.36
Hypertension, n (%)	24 (3.2)	26 (2.9)	0.13	0.72
Heart disease, n (%)[c]	9 (1.2)	3 (0.3)	4.24	0.045
Diabetes mellitus, n (%)	1 (0.1)	5 (0.6)	2.02	0.23
Dyslipidemia, n (%)	12 (1.6)	7 (0.8)	2.42	0.12
Other chronic medical condition, n (%)[d]	13 (1.7)	8 (0.9)	2.31	0.13
Antidepressant, n (%)	60 (8.0)	59 (6.5)	1.25	0.26
Benzodiazepine, n (%)	63 (8.4)	57 (6.3)	2.56	0.11
Mood stabilizer, n (%)	14 (1.9)	8 (0.9)	2.96	0.085
Antipsychotic drug, n (%)	21 (2.8)	18 (2.0)	1.12	0.29
Cardiovascular drug, n (%)	27 (3.6)	26 (2.9)	0.66	0.42

Continuous variables are reported as mean ± standard deviation; categorical variables are listed as numbers (percentage).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GAD, generalized anxiety disorder; HR, heart rate; PSS, Perceived Stress Scale; SBP, systolic blood pressure.
[a] Depressive disorder: major depression, dysthymia, or depressive disorder not otherwise specified; [b] Other anxiety disorder: obsessive-compulsive disorder, panic disorder, or phobia; [c] Heart disease: coronary heart disease, valvular heart disease, or arrhythmia; [d] Other medical condition: asthma, liver disease, kidney disease, thyroid disease, or cancer.

3.3. Moderating effect of age on association of COMT genotypes with resting HF-HRV

Analyzing in the whole sample, the HF index were significantly affected by age ($B = -0.014$, $p = 0.047$) and *COMT rs*4680 genotypes ($B = -0.574$, $p = 0.002$), but this was qualified by a significant age by genotype interaction ($B = 0.014$, $p = 0.001$). The moderating effect of age on the associations between *COMT* Val^{158}Met polymorphism and HF-HRV is illustrated in Fig. 2. In the younger groups [10th (26 years) and 25th (30 years) age percentiles], Met allele carriers exhibited significantly lower HF values ($B = -0.207$, $p = 0.010$; $B = -0.151$, $p = 0.027$, respectively) than homozygous Val-allele carriers. However, the HF measurement became similar across *COMT* genotypes in the average age group [50th (39 years) percentile: $B = -0.024$, $p > 0.05$]. Opposite effects were further found in the older groups [75th (50 years) and 90th (57 years) age percentiles], with Val/Val homozygotes presenting a significantly reduced HF index ($B = 0.131$, $p = 0.049$; $B = 0.230$, $p = 0.010$, respectively) compared to Met allele carriers (Fig. 2A). The Johnson-Neyman technique further revealed that when age ≤ 31.97, Met allele carriers presented significantly lower HF-HRV than Val-allele homozygotes; whereas, when age ≥ 50.04, a significant inversion effect was observed (Fig. 2A).

Furthermore, we also performed a subanalysis in the healthy cohort to test whether the moderation effects of age on the association between *COMT* genotypes and resting HF-HRV exist before the influence of GAD. As gender, BMI, smoking status and PSS scores were significantly related to HF index (Supplementary Table 1), they served as covariates in the subsample analysis. We found that the interaction effect of age and *COMT* on resting HF-HRV was also significant ($B = 0.016$, $p = 0.004$) (Fig. 2). Likewise, in the younger groups (10th and 25th percentiles of age), Met allele carriers had lower resting HF values [$B = -0.175$, $p = 0.038$; $B = -0.127$, $p = 0.082$ (marginally significant), respectively] than homozygous Val-allele carriers. In addition, the Val/Val homozygotes also exhibit significantly lower resting HF-HRV ($B = 0.131$, $p = 0.049$; $B = 0.230$, $p = 0.010$, respectively) compared to Met allele carriers in the older age groups (75th and 90th percentiles of age) (Fig. 2B). The Johnson-Neyman results for the moderation of age on the associations between *COMT* genotypes and resting HF-HRV were presented in Fig. 2B. Finally, even when using only the GAD patients for analysis, the moderation effect remained significant ($B = 0.019$, $p = 0.001$) (data not shown).

Table 2
Correlation between HF-HRV and potential confounders in the whole sample (n = 1655).

Potential covariates	HF-HRV	
	Correlation coefficient (r)	P
Sex (male/female)	−0.103	< 0.001***
BMI	−0.073	0.002**
Current smoking (No/yes)	0.048	0.043*
Weekly regular exercise (Nil/1-2 times per week/ > 2 times per week)	0.007	0.79
PSS	0.015	0.53
Depressive disorder (Without/with)[a]	−0.090	< 0.001***
Other anxiety disorder (Without/with)[b]	−0.052	0.029*
Hypertension (No/yes)	−0.073	0.002**
Heart disease (No/yes)[c]	−0.090	< 0.001***
Diabetes mellitus (No/yes)	−0.065	0.006**
Dyslipidemia (No/yes)	−0.066	0.006**
Other chronic medical condition (No/yes)[d]	−0.069	0.004**
Antidepressant (No/yes)	−0.104	< 0.001***
Benzodiazepine (No/yes)	−0.107	< 0.001***
Mood stabilizer (No/yes)	−0.016	0.50
Antipsychotic drug (No/yes)	−0.093	< 0.001***
Cardiovascular drug (No/yes)	−0.087	< 0.001***

The first category in parenthesis is the reference group.
Abbreviations: BMI, body mass index (kg/m^2); PSS, Perceived Stress Scale; HF-HRV, high-frequency power of heart rate variability.
[a] Depressive disorder: major depression, dysthymia, or depressive disorder not otherwise specified.
[b] Other anxiety disorder: obsessive-compulsive disorder, panic disorder, or phobia.
[c] Heart diseases: coronary heart disease, valvular heart disease, or arrhythmia.
[d] Other medical conditions: asthma, liver disease, kidney disease, thyroid disease, or cancer.

3.4. Effect of resting HF-HRV on the risk of GAD

Using a logistic regression model on the whole sample, adjusted for aforementioned covariates (Table 2), decreased HF-HRV was found to be significantly associated with an increased risk of GAD ($B = -0.278$, $p < 0.001$).

3.5. Indirect prediction of GAD by COMT genotypes via resting HF-HRV

We further examined whether *COMT rs*4680-driven variability in resting HF-HRV was indirectly related to the risk of GAD in an age-dependent way. The model revealed that age and *COMT* variation were significantly negatively correlated with HF index ($\beta = -0.014$;

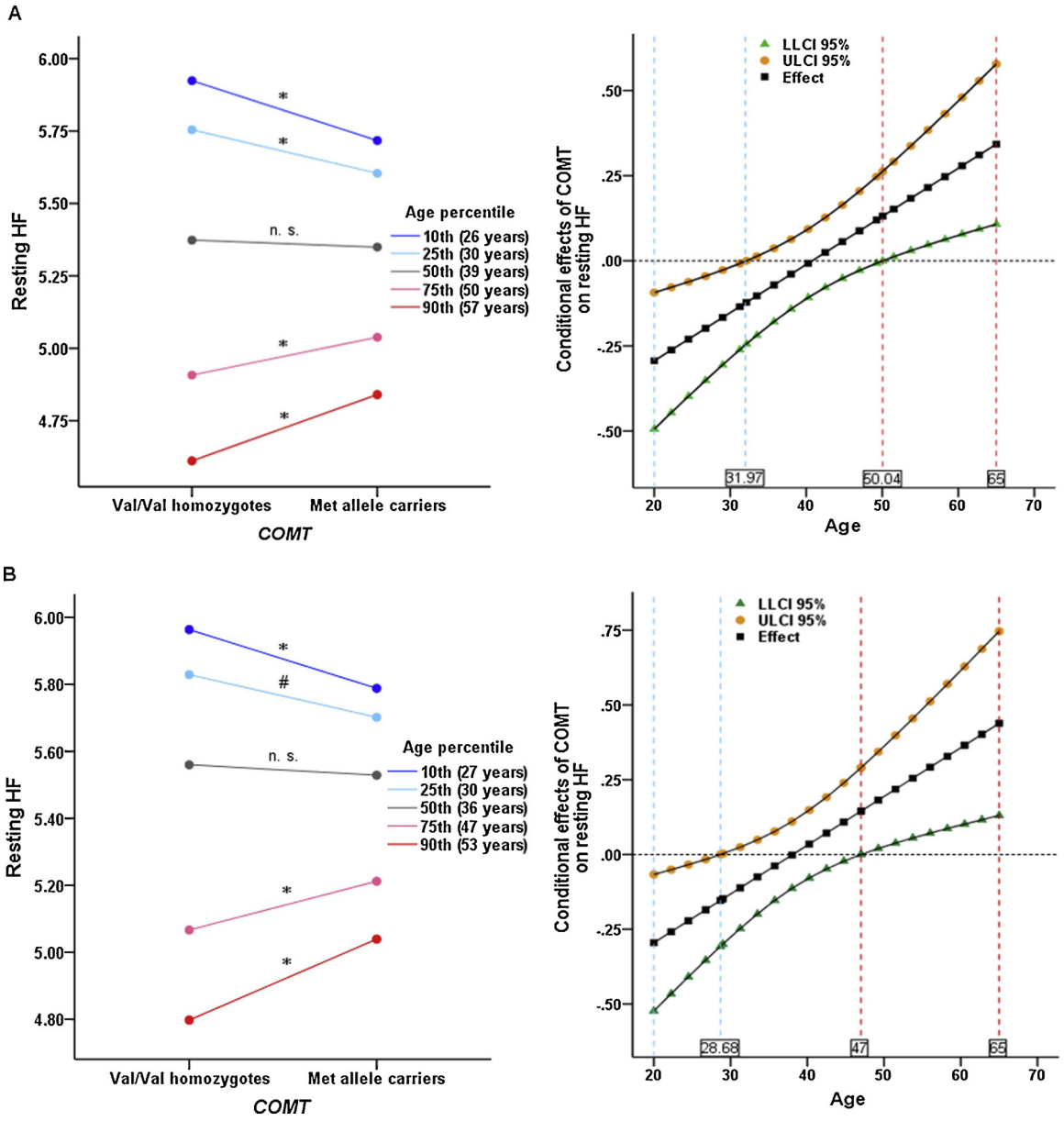

Fig. 2. Association among *COMT* genotypes and resting parasympathetic control. (A) Analyses in the whole sample ($n = 1655$). (B) Analyses in the healthy participants only ($n = 1147$). Data were shown as effects at the 10th, 25th, 50th, 75th, and 90th percentiles of the moderator age (left panel) and conditional effects of age with Johnson-Neyman confidence bands (right panel). The regions between blue dash lines and red dash lines are significant confidence regions (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
*COMT rs*4680 coded as 0 = Val allele homozygotes, 1 = Met allele carriers.
Effect sizes > 0 imply higher resting HF values in the Met allele carriers than Val allele homozygotes. Effect sizes < 0, however, indicate opposite effects.
Abbreviations: *COMT*, Catechol-O-Methyltransferase gene; HF, high-frequency power; LLCI 95% and ULCI 95%, 95% bias corrected lower level confidence interval and upper level confidence interval, respectively (10,000 bootstrap samples).
$^{\#}\ p < 0.1$, $^{*}\ p < 0.05$; n. s.: not significant.

$p = 0.044$; $B = -0.571$; $p = 0.002$), but age × *COMT* genotype was significantly positively correlated with the HF indicator ($B = 0.014$; $p = 0.001$, respectively). The HF-HRV, then, was significantly negatively associated with the risk for GAD ($B = -0.278$; $p < 0.001$) (Fig. 3). Post-hoc analyses showed that the indirect effects of *COMT* on GAD via resting HF were significant in both the younger groups (10th and 25th age percentiles: $B = 0.057$, 95% CI = 0.015˜0.123; $B = 0.041$, 95% CI = 0.007˜0.095, respectively) and the older groups (75th and 90th age percentiles: $B = -0.036$, 95% CI = −0.087˜−0.002; $B = 0.063$, 95% CI = −0.138˜−0.015, respectively), but with opposite patterns (Table 3). When analyzing the *COMT rs*4680 with all three genotypes (coded as 0 for Val/Val, 1 for Val/Met, and 2 for Met/Met; reflecting the presence of the number of Met alleles), the results remained significant (Supplementary Fig. 1). Carriers of two copies of the *COMT* Met allele were associated with the highest risk of GAD via reduced vagal control in the younger participants (10th and 25th age percentiles: $B = 0.054$, 95% CI = 0.016˜0.112; $B = 0.041$, 95% CI = 0.010˜0.088, respectively). However, Val/Val homozygotes are associated with the largest risk of GAD through decreased vagal control at rest in the older participants (90th age percentiles: $B = -0.051$, 95% CI = −0.110˜−0.011) (Supplementary Table 3). Lastly, even when we used the subsample including only drug- and comorbidity-free GAD patients and healthy controls for analyses, adjusted for covariates (gender, BMI, smoking status and PSS levels), the results were still

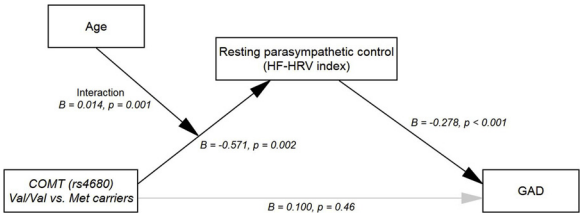

Fig. 3. Age moderates the pathways among *COMT* genotypes, resting parasympathetic control and GAD.
Black arrows represent significant paths and gray arrows indicate non-significant paths. *COMT* rs4680 coded as 0 = Val allele homozygotes, 1 = Met allele carriers.
Abbreviations: *COMT*, Catechol-O-Methyltransferase gene; HF-HRV, high-frequency power of heart rate variability; GAD, generalized anxiety disorder.

Table 3
Conditional indirect effect of *COMT rs*4680 on GAD via decreased resting parasympathetic control at different levels of age.

	Indirect effect	*SE*	LLCI	ULCI
Specific conditional values of age				
10th percentile (26 years)	0.057*	0.027	0.015	0.123
25th percentile (30 years)	0.041*	0.022	0.007	0.095
50th percentile (39 years)	0.007	0.015	−0.020	0.039
75th percentile (50 years)	−0.036*	0.021	−0.087	−0.002
90th percentile (57 years)	−0.063*	0.031	−0.138	−0.015

In the moderated mediation analysis, *COMT rs*4680 was coded as 0 = Val allele homozygotes, 1 = Met allele carriers and GAD was coded as 0 = no GAD diagnosis, 1 = with GAD diagnosis.
Indirect effect sizes > 0 indicate that Met allele carriers are associated with increased risk of GAD via reduced resting vagal control when compared with Val allele homozygotes. However, indirect effect sizes < 0 indicate opposite effects.
Abbreviations: *COMT*, Catechol-O-Methyltransferase gene; GAD, generalized anxiety disorder; *SE*, standard error; LLCI and ULCI, 95% bias corrected lower level confidence interval and upper level confidence interval, respectively (10,000 bootstrap samples).
* $p < 0.05$-based on 95% bias corrected confidence intervals (i.e., not contain the value 0).

significant in both approaches using either two or three *COMT* genotypes (Supplementary Fig. 2).

4. Discussion

The current study was conducted to evaluate the potential age-specific pathways among *COMT* Val^{158}Met variant, resting vagal control and GAD. After adjusting for all the confounders, our main results revealed that Met allele carriers exhibited lower HF-HRV values than Val-allele homozygous carriers in the younger age groups. However, opposite effects were found in the older age groups. Furthermore, in the pathway model, we demonstrated that the interaction of age and *COMT* variant was associated with lower HF-HRV, which in turn was related to increased risk for GAD. The findings here are the first to show that functional *COMT* polymorphism (*rs*4680) is associated with the risk of GAD via altered resting parasympathetic control in an age-dependent manner.

As mentioned previously, dopamine plays a crucial role in reducing prefrontal cortical suppression to the amygdala (Rosenkranz and Grace, 2001) and prefrontal cortex mediated amygdala supression has recently been associated with vagal regulation of HRV (Sakaki et al., 2016). In addition, evidence has shown that blockade of cardiac sympathetic prejunctional beta-2 adrenoceptors that facilitate norepinephrine release can augment reflex vagal control of HR (Kubo et al., 2005). Since COMT is important in determining the levels of catecholamine neurotransmitters (Clark and Noudoost, 2014), and since *COMT rs*4680 effects differ at different ages (Meyer et al., 2016; Smith and Boettiger, 2012); all of these, taken together, support our findings that *COMT rs*4680-driven functional variation may be related to different levels of vagal regulation in an age-dependent manner. Indeed, a previous study has shown that children carrying at least one Met allele of *COMT* represent reduced vagally-mediated HRV responsiveness to stress when compared to those with two Val alleles (Mueller et al., 2012). Our result that the Met allele carriers presented lower resting HF-HRV values than Val-allele homozygotes in younger adult participants may complement the previous research and together indicate that young people with the Met *COMT* allele have decreased parasympathetic function. Moreover, the current study further revealed that older adults showed the opposite pattern, demonstrating Val/Val homozygotes as having a reduction in HF index of HRV. Our intriguing findings were in line with the recent growing evidence for age-dependent gene effects of *COMT rs*4680 on neural functions and related phenotypes, including brain resting state functional connectivity (Meyer et al., 2016), grey matter volume (Rowe et al., 2010), cortical language processing/language ability (Sugiura et al., 2017), and delay reward discounting (Smith and Boettiger, 2012).

As mentioned in the Introduction, the functional Val^{158}Met polymorphism of the *COMT* gene has been reported as a risk factor for GAD and related phenotypes (e.g., anticipatory worry), but with conflicting and confused results. In the current study, considering only the *COMT rs*4680, we did not find an effect of this polymorphism on GAD. However, since neurophysiological endophenotypical markers reflect more proximal effects of genes involved in the development of psychopathology (Gottesman and Gould, 2003), analyzing resting state vagal control, considering also the moderator role of age, may enhance power for genetic discovery to unveil the complex role of *COMT* functional variation in GAD. Indeed, our pathway analysis, in the whole sample, revealed that there was an age-specific correlation of *COMT* Val^{158}Met variant with decreased resting parasympathetic control, which, in turn, was associated with increased risk for GAD. The indirect effects of *COMT* on GAD via resting HF index were significant in both the younger groups and the older groups, with an opposite pattern. In the largest recent genome-wide association study (including all ethnicities), Duncan et al. (2018) have reported no association between *COMT rs*4680 and anxiety-related phenotypes. As the age-dependent *COMT* effects run in the reverse direction, our findings are therefore in line with Duncan et al. (2018) and provide an age-dependent neurophysiological explanation for previous completely conflicting results on associations of *COMT* Val^{158}Met polymorphism with GAD and related phenotypes.

Furthermore, when we analyzed only the healthy subsample, the interaction effects of age and *COMT* on reducing HF-HRV were similar and remained significant, indicating that the interactions were independent of GAD diagnosis. Since decreased vagal tone at rest is associated with GAD, the findings in our healthy cohort suggest that reduced resting parasympathetic control may thus be an endophenotype relevant to GAD rather than the result of the development of the illness. This also provides support for the temporal order of the relationship between decreased resting vagal control and GAD in our pathway model. Notably, the Polyvagal theory, proposed by Porges (2007), has highlighted diminished resting vagal control as the key final common pathway leading to the adverse effects of stress, with the myelinated vagus circuit dampening sympathetically mediated stress responses and fostering calm behavioral states and emotional self-regulation. In line with Porges' perspectives, our results may thus precisely reveal the resting parasympathetic pathway, altered by the age-specific effect of *COMT* gene, linking to GAD.

It is noteworthy that epidemiological studies have demonstrated that some GAD patients have experienced anxiety symptoms in childhood and young adulthood, whereas others have developed their disorder at a later age (especially persons ≥ 50 years of age) (Hoehn-Saric

et al., 1993; Le Roux et al., 2005). Therefore, our results that *COMT* Val^{158}Met polymorphism is associated with the risk of GAD via decreased resting vagal control in an age-specific way may help to explain, at least partly, the underlying neural and genetic mechanisms of a bimodal distribution for age of onset of GAD that peaked in early life and late adulthood (Hoehn-Saric et al., 1993; Le Roux et al., 2005). However, since the ages of our study subjects range only from 20 to 65 years, further studies in adolescents and those aged over 65 years are warranted.

Prior studies have shown that several variables, such as gender, BMI, smoking status, physical exercise, stress levels, medications, and medical and/or psychological morbidities may influence the vagal control of HRV (Cohen et al., 1999). However, our study had a highly-controlled protocol for adjusting the potential effects of the above-mentioned non-genetic confounders. Indeed, even when further analyzing the subsample composed of only drug- and comorbidity-free GAD patients and healthy controls to minimize confounding effects, the results still remained. In addition, HRV patterns may vary in the population due to racial stratification (Martin et al., 2010). However, all the subjects in our study were Han Chinese, drawn from northern Taiwan, which has been revealed to have high levels of genetic homogeneity (Chang et al., 2017). Thus, the current findings may precisely show the associations between the *COMT* gene, resting parasympathetic control and GAD, without the aforementioned biases. Altogether, these facts may suggest that our study results are less likely to be false-positive findings.

This study has some limitations that must be mentioned. We did not control for the respiratory rate, which has been shown to influence HRV measures (Cohen et al., 1999). However, this may not affect our findings since differences in HRV between spontaneous and metronome-guided breathing are relatively small (Bloomfield et al., 2001). Furthermore, it should be cautious when explaining the 5-min changes in the HRV monitoring. However, short-term resting HRV recordings have been demonstrated to be highly reliable (Sandercock et al., 2005). In addition, the studied *COMT* minor (Met) allele frequency is obviously lower in this Asian sample (˜0.25) than in the Western population (˜0.48) (Smith and Boettiger, 2012). Future studies should be conducted in large, racially diverse samples, with an adequately-controlled method such as ours, to validate the current research findings.

5. Conclusion

In summary, our data suggest that functional Val^{158}Met polymorphism of the *COMT* gene is correlated with decreased resting vagal control in an age-dependent manner, and this is, in turn, further associated with increased risk for GAD. These findings may show the age-specific parasympathetic pathways underlying the complex associations between *COMT* Val^{158}Met variant and GAD.

Conflict of interest

None declared.

Contributors

C.C. Chang, H.A. Chang, and W.H. Fang designed the study. C.C. Chang, H.A. Chang, T.C. Chang and W.H. Fang acquired the data, which C.C. Chang, H.A. Chang, W.H. Fang, N.S. Tzeng, Y.P. Liu, F.J. Wan, J.F. Shyu and S.Y. Huang analyzed. H.A. Chang, W.H. Fang and C.C. Chang wrote the article, which all authors critically reviewed and approved for publication.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.03.020.

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