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psychiatric patients, such as those suffering from depression or somatoform pain (Fukunishi et al., Fukunishi

2.3. A achmen e

Attachment components were measured with the Chinese version (Wu et al., 2004) of the 18 item Revised Adult Attachment Scale (RAAS) (Collins, 1996). RAAS is a com monly used scale with established psychometric properties (Collins, 1996); it measures three components underlying adult attachment orientations: the close subscale measures the extent to which an individual is comfortable with close ness and intimacy; the depend subscale measures the extent to which an individual feels he/she can trust and depend on others when needed; and the anxiety subscale assesses the extent to which a individual is worried about being abandoned or unloved by others. Participants were asked to respond to each item on a 5 point Likert scale (1 = not at all characteristic of me, 5 = very characteristic). Each sub scale has 6 items. In this sample, Cronbach s alphas for the close, depend, and anxiety subscales were .58, .56, and .79, respectively. The scoring procedure followed suggestions by Collins (1996).

The Alexithymia test and attachment test were con ducted in groups, with 7 19 participants in each group. The paper and pencil test was conducted firstly for TAS 20 and then for RAAS. Each participant was paid 40 Yuan (about $6) for completing the battery of questionnaires.

2.4. Geno ping

For each participant, we collected 3 5 hairs with hair fol licle cells by sterilized tweezers, cut the hairs into 2 3 cm fragments by a sterile blade, and saved the fragments in a 1.5 ml centrifuge tube. Genomic DNA was extracted from hair follicle cells by Chelex 100 method (de Lamballerie et al., 1994). Considering the relatively low concentration and relatively low purity of the DNA samples we extracted, we employed polymerase chain reaction with single strand conformational polymorphism (PCR SSCP), which has been evidenced to be highly reliable in previous studies (Gong et al., 2014), to genotype the rs6295 polymorphism. C1019G in *5-HT1A* was amplified by PCR. The upstream primer, 5′ TTGTTGTCGTCGTTGTTCGT 3′ and the downstream primer, 5′ ATCGTGTCAGCATCCCAGAG 3′, were recruited. The PCR reaction system contained 2.50 μL 2× reaction MIX (Golden Easy PCR System, TIANGEN), 0.50 μL DNA Template, 2.50 μL ddH_2O, 0.25 μL (25 pmol) upstream primer, and 0.25 μL (25 pmol) downstream primer. A product of 236 bp was amplified with an initial 3 min denaturation at 94 °C, fol lowed by 35 cycles of 94 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. Genotyping was performed by single strand conformation polymorphism (SSCP) method with 13% polyacrylamide gel electrophore sis in 250 V for 40 min and 150 V for 15 h at 4 °C, which was followed by silver staining. On genotyping, six randomly selected samples were sequenced to confirm the alleles of genotyping results. In the current sample of 504 indi viduals, the distribution of genotypes (CC = 316, CG = 165, GG = 23) showed no deviation from Hardy Weinberg Equi librium (χ^2 = .061, p = .805). The genotype frequencies are similar to those found in other Chinese samples (Zhang et al., 2009; Zhou et al., 2013).

3. Re l

3.1. Ale i h mia e

A total score and three subscale scores of TAS 20 were used to examine the effect of genotype on alexithymic charac teristics. Independent samples t test revealed a significant difference in the total score between individuals carry ing the CC genotype (M ± SD: 49.9 ± 8.6) and individuals carrying the CG/GG genotype (51.7 ± 8.6), $t(502) = -2.211$ p = .027, Cohen s d = .20 (Fig. 1A). For the subscales of TAS 20, a 3 (subscales: DIF vs. DDF vs. EOT) × 2 (genotype: CC vs. CG/GG) repeated measures ANOVA revealed a main effect of genotype, $F(1,502) = 4.401$, p = .036. Bonferroni adjusted post hoc t tests revealed that individuals with the CC genotype (17.0 ± 4.4) reported fewer difficulties in identifying feelings on the DIF subscale than those with the CG/GG genotype (18.0 ± 4.2), $t(502) = -2.388$; uncor rected p = .017, Bonferroni adjusted p = .052, Cohen s d = .22 (Fig. 1B). However, the difference on the DDF subscale did not reach significance, CC group (12.8 ± 3.2) vs. CG/GG group (13.1 ± 3.2), $t(502) = -1.089$; uncorrected p = .277, Bonferroni adjusted p = .830, Cohen s d = .10. Neither the difference on the EOT subscale, CC group (20.1 ± 3.2) vs. CG/GG group (20.6 ± 3.4), $t(502) = -1.636$; uncorrected p = .102, Bonferroni adjusted p = .307, Cohen s d = .15.

3.2. A achmen e

Consistent with previous studies (Troisi et al., 2001; Pedrosa Gil et al., 2008), the total score on TAS 20 was negatively correlated with the scores on the RAAS close subscale, $r = -.280$, $p < .001$, and the depend subscale, $r = -.256$, $p < .001$, and positively correlated with the score on the anxiety subscale, $r = .398$, $p < .001$.

For the subscales of RAAS, although ANOVA with sub scales as a within participant factor and genotype as a between participant factor did not find a significant main effect of genotype, $F(1,492) = 1.542$, p = .215, given the previous studies showing that individual differences in attachment orientation are modulated by HTR2A and 5 HTT variations (Caspers et al., 2009; Gillath et al., 2008; Salo et al., 2011), we tentatively conducted t tests for the scores on the three subscales to examine the effect of genotype on attachment orientation. For the close subscale, individuals with the CC genotype (3.62 ± .58) reported higher scores than individuals with the CG/GG genotype (3.51 ± .57), $t(502) = 2.142$; uncorrected p = .033, Bonferroni adjusted p = .098, Cohen s $d = -.19$ (Fig. 1C), indicating that the former seemed to be more comfort able with intimacy than the latter. No difference was found between the two groups on the depend subscale, CC group (3.36 ± .61) vs. CG/GG group (3.33 ± .61), $t(500) = -.609$; uncorrected p = .543, Bonferroni adjusted p = 1.000, Cohen s d = .06, or on the anxiety subscale, CC group (2.50 ± .74) vs. CG/GG group (2.41 ± .77), $t(494) = 1.359$; uncorrected p = .175, Bonferroni adjusted p = .524, Cohen s d = .13.

To make sure that the effects of genotype on alexithymia and attachment orientation survive even when we partial out the potential contributions of environmental factors, we collected data concerning childhood abuse and parental

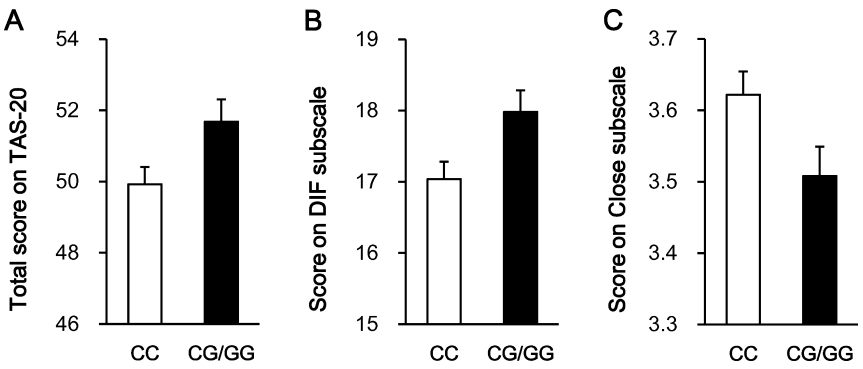

Fig re 1 Effects of *5-HT1A* C 1019G polymorphism on alexithymia and close attachment orientation. (A) Individuals with the CC genotype ($N = 316$) had a lower mean total score on the 20 item Toronto Alexithymia Scale (TAS 20) than individuals with the CG/GG genotypes ($N = 188$). (B) Individuals with the CC genotype reported a lower mean total score on the Difficulty Identifying Feelings (DIF) subscale of TAS 20 than individuals with the CG/GG genotype. (C) Individuals with the CC genotype reported a higher mean score on the close subscale of Revised Adult Attachment Scale than individuals with the CG/GG genotype. Standard errors of the means are shown as error bars.

bonding and entered them as covariates into hierarchical regression (see *Supplementary Materials*). The effects of *5-HT1A* genotype remained to be significant.

4. Di c ion

This study investigated to what extent of C 1019G in *5-HT1A* modulates individuals alexithymic characteristics and attachment orientation. In line with previous studies show ing the increased risk of G alleles in mental disorders (Anttila et al., 2007; Savitz et al., 2009; Kishi et al., 2013), we found that individuals with the G allele showed higher vulnerabil ity to alexithymic symptoms and reported less comfort with intimate relations than individuals with the C allele.

Our findings provided new evidence demonstrating that a serotonin receptor gene is related to the development of alexithymia. A recent study have also shown that individ uals with L/L genotype of 5 HTTLPR in serotonin transporter gene (*5-HTT*) scored higher on TAS 20 and on the DIF sub scale than those with L/S or S/S genotype (Kano et al., 2012). *5-HTT* and *5-HT1A* may play differential roles in the pathology of alexithymia. Although the 5 HTTLPR does not affect the availability of 5 HTT in the living human brain of healthy adult (Murthy et al., 2010), the effects of 5 HTTLPR genotypes on brain function in adults are likely attributable to earlier developmental changes (Frodl et al., 2010; Mueller et al., 2010). During the childhood, the L allele of 5 HTTLPR in *5-HTT* increases the gray matter vol umes in anterior cingulate and amygdale (Pezawas et al., 2005), which are related to the development of alexithymia (Frewen et al., 2006; Radaelli et al., 2014), and leads to a higher risk of alexithymic symptoms (Kano et al., 2012). Unlike the 5 HTTLPR, the C 1019G in *5-HT1A* regulates the serotonin levels in synaptic cleft with the G allele linked to the decreased serotonin level (Lemonde et al., 2003; Albert and Lemonde, 2004; Czesak et al., 2012) and exerts signifi cant effects on the reactivity and volumes of amygdala (Le Francois et al., 2008; Zetzsche et al., 2008). Thus, individual differences in the development of alexithymic characteris tics are partly due to the changes in serotonin levels which are modulated by *5-HT1A*; lower serotonin level in brain is associated with higher risk of alexithymia.

It should be noted, however, that 5 HTT and *5-HT1A* have differential modes in regulating the serotonin level in the brain. 5 HTT, as a hub to inactivate serotonin, distributes on pre synaptic membranes (Zhou et al., 1998) whereas *5-HT1A* receptors, as modulators of serotonin release, distribute on both pre and post synaptic membranes. Stimulating the *5-HT1A* receptors on pre synaptic membranes inhibits the serotonin release from nerve terminals (Fink and Gothert, 2007), thereby lowering the serotonin level in synaptic cleft. Activating 5 HTT on pre synaptic membranes also lowers the serotonin level through enhancing reuptake of serotonin in synaptic cleft (Zhou et al., 1998). However, stimulating the *5-HT1A* receptors on post synaptic membranes can not only inhibit serotonin release from pre synaptic membranes (Hannon and Hoyer, 2008) but also inhibit the activity of post synaptic neurons (Fink and Gothert, 2007). It is plausi ble that alexithymia is also related to *5-HT1A* acting on the post synaptic membranes.

While alexithymia refers to sub clinical inability in iden tifying and describing one s own feelings (Taylor, 1984), the insecure attachment orientation refers to the difficulty in responses to separation from and reunion with others (de Haas et al., 1994; Davila et al., 1997). Nevertheless, previous clinical research showed overlapped psychological symptoms for alexithymia and insecure attachment (Davis et al., 2003; Benetti et al., 2010; Towler and Stuhlmacher, 2013). A previous study showed that a functional polymor phism (T102C, rs6313) in serotonin receptor 2 gene (*5-HT2A*) is associated with the avoidance attachment, such that indi viduals with the TT genotype, relating to higher receptor expression level, scored higher on avoidance than individ uals with CC/CT genotype (Gillath et al., 2008). Consistent with this study, the current study showed that individuals with the GG genotype of *5-HT1A*, relating to higher receptor expression level, reported less comfort with intimate rela tions than individuals with the CG/GG genotype, although this result did not survive Bonferroni correction. Overall, these two studies provide a basis, at the genetic level, for the link between alexithymia and attachment orientation.

Previous studies have shown that early family experiences, such as childhood abuse and parental bonding, are crucial for the development of alexithymia or insecure attachment (Wekerle and Wolfe, 1998; Wearden et al., 2003; De Pan“lis et al., 2008; Pedrosa Gil et al., 2008). Nevertheless, our results showed that the effects of 5-HT1A on alexithymia and attachment still hold after controlling for the effects of these factors. This suggeststhat 5-HT1A may play an important role in regulating the development of alexithymia and attachment orientation independently of early family experiences.

In conclusion, by differentiating individuals according to the polymorphism C-1019G of 5-HT1A and measuring them with the Toronto Alexithymia Scale and the Revised Adult Attachment Scale, we demonstrated for the “rst time the impact of 5-HT1A upon the development of alexithymic characteristics and attachment orientation. Clinical implications of targeting the 5-HT1A receptors as a way to treat alexithymia-related mood disorders may be investigated in further studies.

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Hannon, J., Hoyer, D., 2008. Molecular biology of 5 HT receptors. Behav. Brain Res. 195, 198 213.
Hazan, C., Shaver, P., 1987. Romantic love conceptualized