Reappraisal as a Mediator in the Link Between 5-HTTLPR and Social Anxiety Symptoms

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Social anxiety symptoms have been related to (a) polymorphisms in the serotonin-transporter gene-promoter region (also, serotonin-transporter-linked polymorphic region; 5-HTTLPR) and (b) reduced use of adaptive forms of emotion regulation such as reappraisal. It is not known, however, whether reappraisal functions as a mediator in the link between 5-HTTLPR and social anxiety. To address this issue, 182 unselected community volunteers were tested for 5-HTTLPR status, and self-report measures of social anxiety symptoms and reappraisal use were obtained. Relative to other participants, those with two low-expressing alleles displayed increased social anxiety and decreased reappraisal. As predicted, the influence of 5-HTTLPR on social anxiety symptoms was transmitted via reappraisal, and this effect of 5-HTTLPR was observed using two different measures of reappraisal. These findings suggest that cognitive reappraisal may be an intermediate phenotype of the social anxiety spectrum, and that individuals with low-expressing 5-HTTLPR genotypes may benefit the most from cognitive–behavioral psychotherapy because they do not appear to engage as frequently as others in reappraisal.

Keywords: 5-HTTLPR, emotion regulation, social anxiety, cognitive reappraisal, serotonin

Serotonin Transporter Polymorphisms and Social Anxiety Symptoms

In light of the therapeutic efficacy of selective serotonin reuptake inhibitors (SSRIs) in SAD (van der Linden, Stein, & van Balkom, 2000), and the impact of psychopharmacological manipulations of serotonin availability (e.g., tryptophan depletion) on social anxiety symptoms (Argyropoulos et al., 2004), many genetic association studies have focused on the serotonin-transporter gene (5-HTT). Several functional polymorphisms have been described in the promoter region of this gene (5-HTTLPR), including an insertion/deletion (indel) polymorphism (Lesch et al., 1996) and a single-nucleotide polymorphism (SNP rs25531; Hu et al., 2006; Wendland, Martin, Kruse, Lesch, & Murphy, 2006). The 5-HTTLPR indel polymorphism consists of 14 (the S allele), 16 (the L allele) or more copies (the XL alleles) of a 20–23 base-pair, imperfect repeat sequence (Ehli, Hu, Lengyel-Nelson, Hudziak, & Davies, 2012; Lesch et al., 1996). The SNP rs25531 involves the substitution of an adenine (A) to a guanine (G) in the 6th nucleotide within the first of two extra 20–23 base-pair repeats in the L allele of the indel polymorphism (Hu et al., 2006; Kraft, Slager, McGrath, & Hamilton, 2005; Nakamura, Ueno, Sano, & Tanabe, 2000; Wendland et al., 2006). Therefore, the two resulting L alleles, Lα and Lγ, together with the S allele, comprise a triallelic locus (Hu et al., 2006; Nakamura et al., 2000). The A→G substitution creates a binding site for the transcription factor AP2 (from Activating Protein 2), which suppresses the 5-HTT gene expression in the presence of the Lγ allele and S alleles in dorsal raphe.
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neurons (Hu et al., 2006; Mortensen, Thomassen, Larsen, Whitemore, & Wiborg, 1999). Consequently, the L1 and S alleles are usually expressed together (i.e., carriers of one [S’L’) or two low-expressing alleles [S’S’]) because they reduce the 5-HTT expression with equal magnitude, and then compared with the L1 allele (i.e., L1 homozygotes [L’L’]). Recent studies have shown that the triallelic approach to 5-HTTLPR (i.e., genotyping both polymorphisms, not only the indel) is essential for correctly categorizing genotypes and identifying more reliable associations with phenotypes such as obsessive-compulsive disorder (Hu et al., 2006), therapeutic response to SSRI (Kraft et al., 2005), anterior cingulate volume (Selvaraj et al., 2011), and trait-behavioral inhibition (Whisman, Richardson, & Smolen, 2011).

The S allele has been consistently associated with anxiety-related personality traits (Sen, Burmeister, & Ghosh, 2004), increased attention to negative emotional stimuli (Pergamin-Hight, Bakermans-Kranenburg, van Ijzendoorn, & Bar-Haim, 2012), increased amygdala reactivity to stress (Drabant et al., 2012; Munafo, Brown, & Hariri, 2008), and emotional disorders such as obsessive-compulsive disorder (Lin, 2007) and depression (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Uher & McGuffin, 2010). Recent genetic association studies have also explored the influence of 5-HTTLPR on social anxiety. The low-expressing alleles of 5-HTTLPR polymorphisms (e.g., S or L1) have been significantly associated with symptoms such as state-anxiety escalation and amygdala hyperactivity during public speaking (Furmark et al., 2004), increased shyness and reduced discrimination of angry and neutral faces (Battaglia et al., 2005), and blushing propensity (Domischke et al., 2009) in SAD patients. In addition, these low-expressing alleles also predicted reduced responses to SSRI (Stein, Seedat, & Gelernter, 2006) and placebo treatments that reduce amygdala hyperactivity during public speaking in SAD (Furmark et al., 2008). These converging results seem to support the influence of 5-HTTLPR on key dimensions of SAD, although another recent study found no association between 5-HTTLPR polymorphisms and the SAD diagnosis (Strug et al., 2010). This underscores the possibility that intermediate phenotypes, rather than the global diagnosis of SAD, are associated with 5-HTTLPR polymorphisms.

Emotion Regulation and Social Anxiety Symptoms

Psychological theories have drawn attention to the important role that emotion regulation plays in the onset and maintenance of social anxiety symptoms (Clark & Wells, 1995; Hofmann, 2007). Individuals with social anxiety engage in maladaptive regulatory strategies, such as avoidance and safety behaviors and rumination after the social situation has passed, which leads to maintenance and further exacerbation of social anxiety symptoms (Hofmann, 2007). In addition to making greater use of maladaptive regulation strategies, individuals with social anxiety may make lesser use of adaptive forms of emotion regulation, such as reappraisal, an emotion-regulation strategy that involves changing the meaning of a situation to change the emotional response to that situation (Gillihan et al., 2006; Sheples & Gross, 2011; Szas, Szentagotai, & Hofmann, 2011). Several studies have recently suggested that emotion-regulation difficulties are more specific to SAD than general anxiety disorder (Mennin, McLaughlin, & Flanagan, 2009; Sa-lovey, Stroud, Woolery, & Epel, 2002; Turk, Heimberg, Luterek, Mennin, & Fresco, 2005), in contrast to general symptoms, such as increased negative affect and reduced positive affect, which are shared by most anxiety disorders (Bradley et al., 2011; Kashdan, 2007; Watson, Clark, & Carey, 1988). Therefore, disease-specific interventions have started to target emotion regulation as a key mechanism of change in SAD (Kley, Heinrichs, Bender, & Tuschen-Caffier, 2012; Moscovitch et al., 2012).

Recent studies have identified atypical patterns of emotion regulation in SAD. More specifically, these studies have identified a link between difficulties with reappraisal and social anxiety symptoms (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009; Goldin, Manber, Hakimi, Canli, & Gross, 2009). Both children and adults with SAD use reappraisal less frequently in daily life; they are less able to generate reappraisals of potentially threatening situations; and their self-efficacy concerning reappraisal is reduced, compared with healthy controls (Carthy, Horesh, Apter, Edge, & Gross, 2010; Goldin, Manber-Ball, et al., 2009; Werner, Goldin, Ball, Heimberg, & Gross, 2011). In addition, the lesser down-regulation of negative affect through reappraisal in SAD is related to the severity of social anxiety symptoms, which suggests that the intensity of SAD contributes to emotion dysregulation (Carthy et al., 2010; Goldin, Manber-Ball, et al., 2009). Two functional neuroimaging studies found that SAD patients displayed decreased early recruitment of the brain systems (e.g., dorsolateral prefrontal cortex) implicated in reappraisal of harsh faces or negative self-beliefs (Goldin, Manber-Ball, et al., 2009; Goldin, Manber, et al., 2009). These studies support the view that SAD patients may need more time or more external cues to access and implement reappraisal during social threat. Reappraisal thus appears to be an important predictor of social anxiety symptoms.

5-HTTLPR, Emotion Regulation, and Social Anxiety Symptoms

One crucial question that has not yet been empirically addressed is whether emotion regulation is a mediator in the link between the 5-HTTLPR polymorphisms and social anxiety symptoms. This idea is consistent with the widely discussed hypothesis that emotion regulation may be a candidate intermediate phenotype of emotional disorders (Canli, Ferri, & Duman, 2009; Canli & Lesch, 2007; Hariri & Holmes, 2006; Meyer-Lindenberg & Weinberger, 2006). The main support for this idea came from a functional neuroimaging study that reported reduced functioning of an amygdala-cingulate neural circuit, which is critical for the extinction of negative emotions, in carriers of the S allele of 5-HTTLPR (Pezawas et al., 2005).

Although these findings were replicated and extended by subsequent neuroimaging studies in which emotion regulation was manipulated (Gillihan et al., 2010; Lemogne et al., 2011; but see Firk, Siep, & Markus, 2013; Heinz et al., 2005; Schardeit et al., 2010), there are surprisingly few direct behavioral findings of an association between 5-HTTLPR and individual differences in emotion regulation. Carriers of the low-functioning alleles of 5-HTTLPR have been shown to display reduced emotional resilience (Stein, Campbell-Sills, & Gelernter, 2009) and self-efficacy related to coping with negative emotions (Szily, Bowen, Unoka,
Simon, & Keri, 2008). Three other studies reported higher levels of rumination (Canli et al., 2006; Clasen, Wells, Knopik, McGuey, & Beevers, 2011) and lower levels of reappraisal (Schartd et al., 2010) in S carriers, but a systematic investigation of the association between 5-HTT promoter polymorphisms and specific emotion-regulation strategies is still lacking. In particular, an investigation of the links among 5-HTTLPR, reappraisal, and emotional symptoms is clearly needed.

The Present Study

The goal of this study was to investigate whether the effect of 5-HTTLPR polymorphisms on social anxiety symptoms is transmitted via reappraisal. The sample included a randomly selected set of community-dwelling participants who were screened for social anxiety symptoms using a clinical scale. This allowed us to generalize our findings to a broad segment of the social anxiety spectrum. We predicted that carriers of the low-expressing alleles (i.e., S’S’ and S’L’) of the 5-HTTLPR indel and rs25531 polymorphisms would be associated with increased social anxiety symptoms and reduced use of cognitive reappraisal. Moreover, we hypothesized that cognitive reappraisal would function as a mediator in the link between triallelic 5-HTTLPR genotypes and social anxiety symptoms.

Method

Participants

Participants for this study were 182 (139 women) volunteers. They were all students at Babeş-Bolyai University in Cluj-Napoca, Romania. An a priori sample size analysis had indicated that a minimum of 164 participants were needed (small effect size; $\alpha = .05$; power $\geq 0.8$). Prior to study participation, written informed consent was obtained from all the volunteers. They were all Caucasians of Romanian descent, and came from the same well-circumscribed geographical area. Age ranged from 16 to 42 ($M = 21.3$ years). None of the participants reported cardiovascular or neurological conditions, and they were not on medication (e.g., anxiotytics, beta-adrenergic antagonists, psychotropics). Since our sample was not balanced for sex, all the statistical analyses included sex as covariate in order to control for the effect of this potential confound. All the participants were compensated for their time. The study followed the recommendations of the American Medical Association’s Declaration of Helsinki (World Medical Association, 1964/2008), and it was approved by the Babeş-Bolyai University Research Council. No experimental manipulations were made and all the measures that were administered are described in this section.

Assessment

Social anxiety symptoms were assessed by the total score of the Liebowitz Social Anxiety Scale (LSAS-SR; Fresco et al., 2001; Liebowitz, 1987), which quantifies fear and avoidance in social and performance situations. The participants had to rate the degree to which she or he feared or avoided social situations (e.g., speaking in front of an audience, talking to someone in authority), using a scale from 0 (none/never) to 4 (severe/usually). The internal consistency of LSAS-SR in our sample was excellent (Cronbach’s $\alpha = .9$). LSAS-SR shows good sensitivity and specificity to clinical American Psychiatric Association’s DSM–IV criteria for SAD; a cut-off score of 30 on LSAS-SR correctly identifies over 93% of SAD patients (Mennin et al., 2002), but studies on student samples emphasize that scores above 55 more reliably indicate moderate and severe social anxiety (Russell & Shaw, 2009). Although these clinical cut-offs were informative regarding possible SAD diagnoses in our participants, a clinical LSAS-SR score was not an inclusion criterion.

One of the measures of individual differences in emotion regulation was the cognitive reappraisal score from the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003). Using a scale from 1 (strongly disagree) to 7 (strongly agree), the participants rated their habitual use of reappraisal (e.g., when I want to feel less negative emotion, I change what I’m thinking about) and suppression (e.g., I keep my emotions to myself). The internal consistency of ERQ reappraisal in our sample was very good (Cronbach’s $\alpha = .84$). Because individual differences in cognitive reappraisal were the focus of this study, we used a second, convergent measure of reappraisal from the Cognitive Emotion Regulation Questionnaire (CERQ; Garnefski, Kraaij, & Spinhoven, 2001). In this questionnaire, the participants rated on a scale from 1 (almost never) to 5 (almost always) the frequency of using reappraisal when confronted with negative events (e.g., I look for the positive sides to the matter). The internal consistency of CERQ reappraisal in our sample was good (Cronbach’s $\alpha = .72$). The significant correlation between ERQ reappraisal and CERQ reappraisal scores (Pearson $r = .33$, $p = .005$) in the present sample confirmed that we could use these scales as parallel measures of the frequency with which cognitive reappraisal is used. ERQ expressive suppression scores (Cronbach’s $\alpha = .76$) were used in additional analyses designed to explore the specificity of cognitive reappraisal as a mediator.

Genotyping

DNA was extracted from leukocytes (ethylenediaminetetraacetic acid; EDTA-anticoagulated blood) using the Genomic DNA Extraction Kit (Fermentas, Vilnius, Lithuania) and kept at $-20^\circ C$. Both 5-HTTLPR indel and rs25531 genotyping were performed using published protocols (Miu et al., 2012; Vulturar, Chis, Ungureanu, & Miu, 2012). Briefly, the polymerase chain reaction (PCR) assay conditions were optimized as follows: Each reaction was carried out in a 25-$\mu$L volume: 50 ng of genomic template, 12.5-$\mu$l PCR mastermix (2x); the forward primer (5’-GGCGGTGCGCCCTCTGAATGC-3’) and reverse primer (5’-GAGGGACTGAGCTGGACACAC-3’) from Generi-Biotech (Hradec Kralove, Czech Republic) were used to amplify a region encompassing 5-HTTLPR. These primers yield amplicons of 529 (for the L allele) or 486 base pairs (for the S allele). Thermal cycling consisted of 3 min of initial denaturation at $94^\circ C$ followed by 31 cycles of $94^\circ C$ (40 s), 57 $^\circ C$ (40 s) and 72 $^\circ C$ (40 s), each with a final extension step of 4 min at 72 $^\circ C$. The $L_S$ and $L_A$ alleles of rs25531 were subsequently studied by enzymatic digestion of 10 $\mu$l of PCR products that were digested by HpaII. This restriction enzyme recognizes and cuts a 5’-C/C/GG-3’ sequence resulting in the following fragments: 340 base pairs, 127 base pairs and 62 base pairs.
pairs for the $L_A$ allele; 174, 166, 127 and 62 base pairs for the $L_g$ allele; 297 base pairs, 127 base pairs and 62 base pair for the $S_g$ allele; and 166, 131, 127 and 62 base pairs for the $S_g$ allele. Finally, 10 μl of remaining PCR product and 15 μl of restriction-enzyme assay solution were loaded onto a 2.5% agarose gel and visualized by ethidium bromide for size estimation. The 5-HTTLPR allele frequencies were 0.44 for the $S_g$ allele, 0.48 for the $L_A$ allele and 0.07 for the $L_g$ allele, similar to the ones that were reported for Caucasians by Hu et al. (2006). One of the participants carried an XL (>16 repeats) allele (Ehli et al., 2012). The genotypes were categorized into $S'S'$ (i.e., carriers of two low-expressing alleles, SS, $L_gL_g$, $S_gS_g$; N = 50); $S'L'$ (i.e., carriers of one low-expressing allele, $S_gL_A$, $L_gS_g$; N = 81); and $L'L'$ (i.e., carriers of two high-expressing alleles, $L_AL_A$, $L_gL_g$; N = 51). These genotypes were in Hardy-Weinberg equilibrium ($\chi^2 = 2.2, n.s.;$ Rodriguez, Gaunt, & Day, 2009). There were no age differences between the genotypes.

**Data Analysis**

There were no missing data. Analyses of covariance (ANCOVA), with sex as covariate, were used to test for differences between genotypes in reappraisal, suppression, and in social anxiety symptoms. There were no sex differences in ERQ reappraisal, CERQ reappraisal, ERQ suppression, or LSAS-SR scores (all $p > .05$). Mediation was tested using multiple regression (Baron & Kenny, 1986; Frazier, Tix, & Barron, 2004; Hoyt, Imel, & Chan, 2008), following the steps suggested by Baron and Kenny (1986); see Figure 1: (1) the initial variable (genotype group) was significantly related to the outcome variable (LSAS-SR score; Path c); (2) the initial variable was related to the mediator (emotion-regulation score; Path a); and (3) the mediator was significantly associated with the outcome variable when regressed on both the mediator and the initial variable (Path b), and the effect of the initial variable (Path c') was reduced compared with that (Path c) in the first regression. Because there is no general consensus on the additive or dominant status of the low-expressing alleles of 5-HTTLPR, the genotype was coded according to the number of $S$' alleles present (instead of dummy coding comparing low-expressing alleles carriers with $L'$ homozygotes: 0 = $L'L'$; 1 = $S'L'$; and 2 = $S'S'$). For similar approaches, see Conway et al., 2012; Heinz et al., 2005. We tested the significance of the indirect effect of 5-HTTLPR on social anxiety through emotion regulation using the bootstrapping method (bias corrected, with 1,000 iterations; Preacher & Hayes, 2004; Shrout & Bolger, 2002). The indirect effect was significant if the CI from bootstrapping did not include zero (Frazier et al., 2004; Shrout & Bolger, 2002). Two sets of analyses were conducted, one for each of the two reappraisal measures (i.e., ERQ, CERQ). In addition, we tested an alternative model in which ERQ-suppression was specified as a mediator in the relation between 5-HTTLPR and social anxiety symptoms. This allowed us to explore the specificity of cognitive reappraisal as a mediator. All the analyses, including the bootstrapping texts, were run in SPSS.

**Results**

**5-HTTLPR and Social Anxiety Symptoms**

Table 1 presents the scores of participants from each genotype group on anxiety symptoms (LSAS-SR). Participants with LSAS-SR scores over 55 represented 49% ($N = 89$), categorized using Russell and Shaw’s (2009) cut-off scores as follows: Moderate social anxiety (Scores 55–64; 20%); marked social anxiety (Scores 65–79; 14%); severe social anxiety (Scores 80–95; 10%); and very severe social anxiety (Scores 96+: 4%). An ANCOVA, with 5-HTTLPR genotype group as between-subjects factor and sex as covariate, indicated a significant effect of 5-HTTLPR on LSAS-SR scores, $F(2, 179) = 20.32, p < .01, \eta^2_p = 0.28^1$. The $S'S'$ group showed significantly higher LSAS-SR scores than the $S'L'$ and $L'L'$ groups.

**5-HTTLPR and Emotion Regulation**

Table 1 also shows reappraisal scores from ERQ and CERQ by genotype group. An ANCOVA, with the 5-HTTLPR genotype group as between-subjects factor and sex as covariate, indicated that genotype had a significant effect on the ERQ-reappraisal scores, $F(2, 179) = 15.8, p < .01, \eta^2_p = 0.32$. The $S'S'$ group displayed significantly lower ERQ-reappraisal scores than both the $S'L'$ ($p < .01$) and the $L'L'$ groups ($p < .01$). The difference between the $S'L'$ and $L'L'$ genotypes on ERQ-reappraisal scores was not significant ($p = .99$). The significant effect of the 5-HTTLPR genotype was also evident on reappraisal scores from CERQ, $F(2, 179) = 8, p < .01, \eta^2_p = 0.19^2$. The $S'S'$ group had significantly lower CERQ-reappraisal scores than the $L'L'$ group ($p < .01$), and the $S'L'$ group had significantly lower scores than the $L'L'$ group ($p < .01$). The difference between the $S'S'$ and $S'L'$ groups was not significant ($p < .5$). ERQ-suppression scores were not significantly different between genotype groups (see Table 1).

**5-HTTLPR, Reappraisal, and Social Anxiety Symptoms**

Table 2 presents the regression coefficients from the analyses of the mediation model, in which the 5-HTTLPR genotype was the initial variable, LSAS-SR was the outcome variable, and the ERQ-reappraisal was the potential mediator (see also Figure 1A).

To test the direct effect (Path c in Figure 1A), LSAS-SR scores were first regressed on the genotype. As hypothesized, the genotype had a significant effect on the ERQ-reappraisal scores ($p < .01, \eta^2_p = 0.28$). The effects of age and Age $\times$ 5-HTTLPR on LSAS-SR scores were not significant. The $S'L'$ group showed significantly higher LSAS-SR scores than the $S'L'$ and $L'L'$ groups.

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1. The effect of genotype remained significant and the effect size was comparable even after excluding the covariate (i.e., sex) from the analysis, $F(2, 179) = 13.18, p < .01, \eta^2_p = 0.28$. The effects of age and Age $\times$ 5-HTTLPR on LSAS-SR scores were not significant.

2. Excluding the covariate from the analyses did not change the effects of genotype on ERQ reappraisal, $F(2, 179) = 15.73, p < .01, \eta^2_p = 0.31$ and CERQ reappraisal, $F(2, 179) = 7.46, p < .01, \eta^2_p = 0.18$. Similarly, excluding the covariate from the analyses on ERQ-suppression scores did not change the null result, $F(2, 179) = 0.41, p > .05$.
anxiety was regressed on both the genotype and the reappraisal scores. Reappraisal and social anxiety were significantly related while controlling for the genotype (Path b in Figure 1). The CI [5.63, 19.8] from bootstrapping did not include zero, which confirmed that CERQ reappraisal was a significant mediator between the genotype and social anxiety. The indirect effect was also significant when we restricted the analyses to the participants who scored above 55 on LSAS: the CI from bias-corrected bootstrapping was [2.26; 14.41]. A reverse-causality analysis that tested the indirect effect of the genotype on CERQ reappraisal through social anxiety revealed no significant mediation, CI [−1.19, 0.04] from bias-corrected bootstrapping.

To examine the specificity of reappraisal as a mediator, we tested whether ERQ suppression mediated the relation between 5-HTTLPR and social anxiety symptoms (see Table 4). The 5-HTTLPR was not related to ERQ suppression (Path a in Figure 1C), and suppression and social anxiety were not significantly related while controlling for the genotype (Path b in Figure 1C). The indirect effect was not significant, as indicated by the CI [−3.35; 4.93] from bootstrapping.

Discussion

Low-expressing 5-HTTLPR genotypes were associated with decreased reappraisal and increased social anxiety. Using two measures of cognitive reappraisal, we found converging evidence that individual differences in this emotion-regulation strategy carry the influence of 5-HTTLPR genotype on anxiety symptoms. By contrast, expressive suppression was not linked to 5-HTTLPR and social anxiety. These findings support the hypothesis that 5-HTTLPR contributes to social anxiety symptoms via decreased reappraisal.

Cognitive Reappraisal Is an Intermediate Phenotype

Individual differences in reappraisal are known to influence levels of positive and negative affect, as well as social support and well-being in the general population (Gross & John, 2003; Sheppes & Gross, 2011). In contrast, decreased use of such adaptive emotion-regulation strategies in daily life significantly contributes to the development of psychopathology (Aldao, Nolen-Hoeksema, & Schweizer, 2010; Bradley et al., 2011; Szasz et al., 2011), including social anxiety symptoms (Rusch, Westermann, & Lincoln, 2011). In a diathesis-stress framework, it has been hypothesized that the risk of social anxiety problems, associated with the decreased use of cognitive reappraisal, is related to a certain genetic background. However, until now, there were no studies that traced the pathway from genetic susceptibilities to social anxiety, through emotion regulation. The present study found that the habitual use of cognitive reappraisal significantly mediated the link between the 5-HTTLPR genotype and social anxiety symptoms.

3 Based on Preacher and Kelley (2011), we estimated the effect size as the ratio of the obtained indirect effect to the maximum possible indirect effect: \( k^2 = 0.27 \).

4 The effect-size index (Preacher & Kelley, 2011) for this indirect effect is \( k^2 = 0.09 \).
This finding complements previous neuroimaging reports of an association between 5-HTTLPR and activity in an amygdala-cingulate circuit related to emotion regulation (Firk et al., 2013; Heinz et al., 2005; Pezawas et al., 2005; Schardt et al., 2010). In the largest of these studies (N = 94), S-allele carriers displayed reduced functional connectivity between the rostral subgenual portion of the anterior cingulate cortex and the amygdala during perception of fearful stimuli (Pezawas et al., 2005). The reduced correlation of activity in this cingulate-amygdala circuit predicted almost 30% of the variance in dispositional anxiety (Pezawas et al., 2005). However, in a similar study, Heinz et al. (2005) reported an increased functional connectivity between the ventromedial prefrontal cortex and the amygdala during the perception of aversive images in S-allele carriers. By manipulating emotion regulation, two recent studies also found that emotional detachment (Schardt et al., 2010) or cognitive reappraisal (Firk et al., 2013) with aversive images triggered increased activity in brain areas associated with the top-down control of emotions (e.g., ventrolateral and dorsolateral prefrontal cortex; left medial prefrontal cortex; rostral anterior cingulate cortex) in S-carriers.

As pointed out by Pezawas et al. (2005), the differences between these neuroimaging results may be explained by the focus on aversive images in S-allele carriers. By manipulating emotion regulation, two recent studies also found that emotional detachment (Schardt et al., 2010) or cognitive reappraisal (Firk et al., 2013) with aversive images triggered increased activity in brain areas associated with the top-down control of emotions (e.g., ventrolateral and dorsolateral prefrontal cortex; left medial prefrontal cortex; rostral anterior cingulate cortex) in S-carriers.

This stress-coping mismatch hypothesis is based on animal studies, and it has started to be supported in 5-HTTLPR knockout rodents exposed to escapable or inescapable stress (van der Doelen, Kozicz, & Homberg, 2013). Based on the recent findings

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Liebowitz social anxiety scale</th>
<th>Cognitive reappraisal (Emotion regulation questionnaire)</th>
<th>Positive reappraisal (Cognitive emotion regulation questionnaire)</th>
<th>Expressive suppression (Emotion regulation questionnaire)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L’L’</td>
<td>44.84 ± 4.07</td>
<td>5.41 ± 0.06</td>
<td>16.47 ± 0.66</td>
<td>2.77 ± 0.31</td>
</tr>
<tr>
<td>S’L’</td>
<td>49.78 ± 3.86</td>
<td>5.39 ± 0.07</td>
<td>13.6 ± 0.61**</td>
<td>3.09 ± 0.21</td>
</tr>
<tr>
<td>S’S’</td>
<td>76.57 ± 5.02**</td>
<td>4.9 ± 0.06**</td>
<td>12.47 ± 0.76**</td>
<td>3.05 ± 0.26</td>
</tr>
</tbody>
</table>

Note. Values in cells are M ± 1 SEM.

** p < .01.
that people choose certain emotion-regulation strategies depending on the intensity of stress (i.e., reappraisal for low-intensity stress vs. distraction for high-intensity stress; Sheppes, Scheibe, Suri, & Gross, 2011; Sheppes et al., 2012), and that like 5-HTT knockout rodents, people of the SS genotype of 5-HTTLPR show increased sensitivity to stress (Drabant et al., 2012), the following predictions could be made in humans, in line with the stress-coping mismatch hypothesis: (a) Exposure to lower or higher levels of stress during childhood would be specifically associated with higher habitual reappraisal and distraction, respectively; (b) emotional problems in adults would be increased in habitual reappraisers who were exposed to low levels of stress during childhood, but currently experience high levels of stress, as well as in habitual distractors who were exposed to high levels of stress during childhood, but currently experience low levels of stress; and (c) the latter effect would be facilitated in S homozygotes for 5-HTTLPR. These interesting predictions remain to be tested in future studies (ideally, longitudinal studies); they also offer an explanation for other emotion-regulation strategies (e.g., distraction) and the similarity between the early and adult levels of stress.

### Implications for Psychotherapy

The present study has important implications for psychotherapy. Cognitive–behavioral therapy (CBT) for anxiety and depression has focused on teaching reappraisal skills (Clark & Beck, 2010; Mennin, Ellard, Fresco, & Gross, 2013). Recent studies have demonstrated that the successful acquisition of these skills differentiated between responders and nonresponders to CBT, and correlated with decreases of symptoms in SAD (Heinz et al., 2005; Kley et al., 2012; Moscovitch et al., 2012). Moreover, 5-HTTLPR was also found to influence the response to CBT in a large sample of children with anxiety disorders (Eley et al., 2012). The SS genotype was associated with increased response to therapy, in comparison with SL and LL genotypes (Eley et al., 2012). Based on the present finding that low-expressing 5-HTTLPR genotypes are also associated with decreased use of cognitive reappraisal, and in light of the observation that cognitive reappraisal is a key mechanism of change in CBT, we suggest that the patients with the low-expressing 5-HTTLPR genotypes may ben-

### Table 3

**Results of Multiple Regression Analyses on the Mediator Role of CERQ Positive Reappraisal in the Relationships Between 5-HTTLPR Genotype and Social Anxiety Symptoms**

<table>
<thead>
<tr>
<th>Testing steps in mediation model</th>
<th>B</th>
<th>SE B</th>
<th>95% CI</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing step 1 (Path c)</td>
<td></td>
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<tr>
<td>Outcome: LSAS social anxiety</td>
<td></td>
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<tr>
<td>Predictor: Genotype (L’L’ vs. S’L’ vs. S’S’)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.86</td>
<td>3.5</td>
<td>9.39, 22.52</td>
<td>0.48**</td>
</tr>
<tr>
<td>Testing step 2 (Path a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: CERQ-reappraisal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predictor: Genotype</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Testing step 3 (Paths b and c')</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: LSAS social anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediator: CERQ-reappraisal (Path b)</td>
<td>−1.51</td>
<td>0.76</td>
<td>−2.78, −0.13</td>
<td>−0.22*</td>
</tr>
<tr>
<td>Predictor: Genotype (Path c')</td>
<td>12.85</td>
<td>3.27</td>
<td>5.9, 19.93</td>
<td>0.39**</td>
</tr>
</tbody>
</table>

<sup>a</sup> = L’L’ (i.e., L<sub>A</sub>L<sub>A</sub> and L<sub>B</sub>Y/XL genotypes); <sup>1</sup> = S’L’ (i.e., S/L<sub>A</sub> and L<sub>C</sub>/L<sub>A</sub> genotypes); <sup>2</sup> = S’S’ (S/S, L<sub>C</sub>/L<sub>A</sub> and S/L<sub>C</sub> genotypes).

* p < .05. ** p < .01.

### Table 4

**Results of Multiple Regression Analyses on the Mediator Role of ERQ Expressive Suppression in the Relationships Between 5-HTTLPR Genotype and Social Anxiety Symptoms**

<table>
<thead>
<tr>
<th>Testing steps in mediation model</th>
<th>B</th>
<th>SE B</th>
<th>95% CI</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing step 1 (Path c)</td>
<td></td>
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<td></td>
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<tr>
<td>Outcome: LSAS social anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predictor: Genotype (L’L’ vs. S’L’ vs. S’S’)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.86</td>
<td>3.5</td>
<td>9.39, 22.52</td>
<td>0.48**</td>
</tr>
<tr>
<td>Testing step 2 (Path a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: ERQ-suppression</td>
<td></td>
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<tr>
<td>Predictor: Genotype</td>
<td></td>
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</tr>
<tr>
<td>Testing step 3 (Paths b and c')</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome: LSAS social anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediator: ERQ-suppression (Path b)</td>
<td>0.96</td>
<td>2.11</td>
<td>−3.25, 5.19</td>
<td>0.04</td>
</tr>
<tr>
<td>Predictor: Genotype (Path c')</td>
<td>15.73</td>
<td>3.53</td>
<td>8.67, 22.79</td>
<td>0.47**</td>
</tr>
</tbody>
</table>

<sup>a</sup> = L’L’ (i.e., L<sub>A</sub>Y/L<sub>A</sub> and L<sub>B</sub>/L<sub>A</sub> genotypes); <sup>1</sup> = S’L’ (i.e., S/L<sub>A</sub> and L<sub>C</sub>/L<sub>A</sub> genotypes); <sup>2</sup> = S’S’ (S/S, L<sub>C</sub>/L<sub>A</sub> and S/L<sub>C</sub> genotypes).

* p < .05.

Note. Abbreviations: B = unstandardized regression coefficient; β = standardized regression coefficient; CI = confidence interval; SE = standard error.

This article is intended solely for the personal use of the individual user and is not to be disseminated broadly.
efit the most from CBT because their cognitive reappraisal skills are markedly deficient. Therefore, our findings may have timely implications for the new field of “therapygenetics” (Beeevers & McGeary, 2012; Eley et al., 2012), by highlighting the role of cognitive reappraisal in the link between 5-HTTLPR and response to CBT.

Limitations and Future Directions

Although promising, the present study has several limitations. One is the relatively modest sample size. Recent meta-analyses have failed to replicate Gene × Environment interaction effects (e.g., 5-HTTLPR × Child Abuse on depression), which have been reported in samples too underpowered to detect small genetic effects (Duncan & Keller, 2011; Risch et al., 2009). These results highlight the importance of the sample size in reducing the probability of Type I errors, which would require over a thousand participants to be recruited for detection of a genetic influence with small effect size (Duncan & Keller, 2011). However, similar, more inclusive meta-analyses (Karg, Burmeister, Shedden, & Sen, 2011; Uher & McGuffin, 2010), i.e., studies with alternative measures or statistical models were considered, replicated the original effects. Although there is general agreement that results from genetic association studies with underpowered samples should be taken with caution until they are replicated in large-scale studies or meta-analyses (Caspi et al., 2010), it has also been acknowledged that the rate of false positive findings may not be that high in these studies (Lohmueller, Pearce, Pike, Lander, & Hirschhorn, 2003). Large-scale genetic association studies may offer a sample-size standard that has seldom been achieved in psychological research, and the solution of organizing multicentric studies may be problematic because of ethnic stratification and increased variability of the 5-HTTLPR allele distribution in different populations, for instance (Murdoch, Speed, Pakstis, Heffelfinger, & Kidd, 2013). Even though they do not offer definitive results, hypothesis-driven genetic association studies such as this one, i.e., those with explicitly defined phenotypes, precise localization of polymorphisms, low genotyping error rate, availability of genotype counts and analyses, that avoid overlap with previous studies (Buckland, 2001; Conneally & Sparkes, 1998; Lohmueller et al., 2003), may guide the discovery of true genetic influences on psychological phenotypes. Notwithstanding these favoring perspectives, we acknowledge that our promising results should be independently replicated before they begin to influence mainstream research on social anxiety.

A second limitation of the present study is the lack of clinical diagnosis of the participants. Previous genetic studies failed to find an association between 5-HTTLPR and the SAD diagnosis (Strug et al., 2010), but identified significant influences of this genotype on particular phenotypes that characterize the social anxiety spectrum. This motivated our decision to focus on social anxiety symptoms in nonpatient volunteers, which allows us to generalize our findings to a broad segment of the social anxiety spectrum. However, it must be acknowledged that the generalization of the present findings might be limited by the underrepresentation of men in this sample.

A third important limitation of this study is that we focused on just one form of emotion regulation and just one genetic susceptibility factor. Our decision to focus on one form of emotion regulation—reappraisal—using two different measures allowed us to show that our core findings were robust in that they were evidenced using two different measures of reappraisal. However, we explored the specificity of reappraisal by showing that suppression was not a mediator. Our decision to focus on just one genetic susceptibility factor was motivated by the fact that prior research strongly pointed to this risk factor as a promising candidate susceptibility factor. One important direction for future research might be to investigate the additive effects of 5-HTTLPR and Tryptophan Hydroxylase 2 gene polymorphisms (i.e., rs4570625), which were both related to emotion appraisal (this study: Szily et al., 2008; Szily & Keri, 2012) and are known to interactively affect other emotion phenotypes (Herrmann et al., 2007). In the present study, the frequency of using reappraisal or suppression was assessed using self-report instruments (i.e., ERQ and CERQ), which, however reliable, are subject to demand characteristics and limited to emotion regulation processes to which participants have introspective access. Therefore, future studies could also measure other aspects (e.g., ability rather than frequency) and processes of emotion regulation (e.g., automatic rather than voluntary), using performance-based and neural assessments (Firk et al., 2013; Schardt et al., 2010). Moreover, other clinically relevant forms of emotion regulation (e.g., reappraisal vs. distraction) that may be adaptive or maladaptive depending on the level of stress during development and adulthood (Clasen et al., 2011; Homberg, 2012), could also be investigated in the future.

The present study supported the hypothesis that reappraisal is a potential intermediate phenotype on the pathway from 5-HTTLPR to social anxiety symptoms. These results have important implications for diathesis-stress theories of SAD, by identifying 5-HTTLPR as a genetic candidate that influences social anxiety symptoms through cognitive reappraisal, and for clinical interventions that include an emphasis on cognitive reappraisal training, such as CBT.

References


