RESEARCH ARTICLE

The interaction of *5-HTT* variation, recent stress, and resilience on current anxiety levels in adolescents and young adults from the general population

Theresa M. Ollmann[1] | Catharina Voss[1] | John Venz[1,2] | Esther Seidl[1] |
Jana Hoyer[1] | Hanna Kische[1] | Lars Pieper[1,2] | Miriam A. Schiele[3] |
Katharina Domschke[3,4] | Katja Beesdo-Baum[1,2]

[1]Institute of Clinical Psychology and Psychotherapy, Behavioral Epidemiology, Technische Universität Dresden, Dresden, Germany

[2]Institute of Clinical Psychology and Psychotherapy, Center for Clinical Epidemiology and Longitudinal Studies (CELOS), Technische Universität Dresden, Dresden, Germany

[3]Department of Psychiatry and Psychotherapy, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[4]Faculty of Medicine, Center for Basics in NeuroModulation, University of Freiburg, Freiburg, Germany

Correspondence
Theresa M. Ollmann, Institute of Clinical Psychology and Psychotherapy, Behavioral Epidemiology and Center for Clinical Epidemiology and Longitudinal Studies, Technische Universität Dresden, Chemnitzer St, 46 01187 Dresden, Germany.
Email: theresa_magdalena.ollmann@tu-dresden.de

Funding information
German Federal Ministry of Education and Research, Grant/Award Numbers: 01ER1303, 01ER1703

Abstract

Background: Previous work on gene-environment (GxE) interplay concerning anxiety has focused on the interaction of *5-HTT*LPR with childhood adversities or traumatic events whereas the impact of recent stressors is understudied, as is the integration of resilience. The current study aimed to investigate the interactive effect of *5-HTT*LPR and recent stress on anxiety in adolescents considering resilience as buffer of a GxE risk constellation.

Method: In a random population-based sample of 14–21 years old from Dresden, Germany, (N = 1180; genotyped = 942) recent stress (Daily Hassles [DH] Scale, Perceived Stress Scale, Screening Scale of the Trier Inventory for the Assessment of Chronic Stress), resilience (Connor–Davidson resilience scale) and anxiety (Patient Reported Outcome Measurement Information System Anxiety Short Form) were assessed via questionnaire in 2015 or 2016.

Results: Fractional regression models revealed that resilience interacted with recent stress in form of DH as well as recent chronic stress and *5-HTT*LPR regarding anxiety. Participants carrying the more active $L_A L_A$ genotype reported consistently higher levels of anxiety when experiencing more DH or more recent chronic stress and having low levels of resilience. When the resilience scores were high, $L_A L_A$ carriers reported the lowest anxiety scores despite DH or recent chronic stress.

Conclusion: Findings revealed an interactive relationship between 5-*HTT*LPR genotype and recent stress suggesting resilience to function as an additional dimension buffering the impact of a GxE risk constellation. Early interventions to build resilience may be useful to prevent an escalation of distress and associated unfavorable health outcomes.

KEYWORDS
5-*HTT*LPR, adolescence, anxiety, epidemiology, genetics, resilience, stress

1 | INTRODUCTION

Anxiety is usually adaptive in response to potentially harmful or threatening situations, but may become pathological when being out of proportion to the actual danger posed, when occurring frequently and persistently, and when leading to individual suffering or impairment (APA, 2013). Just as anxiety, stress is inevitably embedded in our life and has a pivotal impact on health. It is well-known that stress in form of major stressors, for example, childhood adversities or (non-) traumatic life events, are associated with numerous psychopathological symptoms and mental disorders including anxiety (for a review see Klauke, Deckert, Reif, Pauli, & Domschke, 2010). Further, research indicates that also recent stress, such as ongoing daily hassles (DH) or recent chronic stress in everyday life might increase the risk for anxious mood (D'Angelo & Wierzbicki, 2003), anxiety symptoms (Barrett & Heubeck, 2000; Kanner et al., 1981) and anxiety disorders (Asselmann et al., 2017). DH comprise everyday demands and conditions perceived as irritating, frustrating or stressful (Lazarus, 1986), whereas recent chronic stress is marked by a creeping onset, prevalent long-lasting recurring stressors with uncontrollable consequences. Thus, DH or recent chronic stress occurs much more frequently than major life events, having a substantial effect regarding anxiety.

However, people differ in their susceptibility to recent stress, and susceptibility to stress may have biological roots, primarily in the serotonergic system, reflected by an interaction of stress with genetic variation on anxiety. The serotonin transporter gene linked polymorphic region (5-HTTLPR), located in the promoter region of the serotonin transporter gene (SCL6A4; chromosome 17q12) has a key role within serotonergic neurotransmission by regulating the serotonin reuptake from the synaptic cleft (Lesch, Zeng, Reif, & Gutknecht, 2003). The polymorphism comprises a short allele (S), associated with less transcription of the serotonin transporter, compared with the long allele (Lesch, Bengel et al., 1996). A single-nucleotide polymorphism rs25531 (A>G) within the long allele renders the L_G allele functionally equivalent to the S allele (i.e., reduced 5-HTT availability), while the A allele results in increased 5-HTT expression (Hu et al., 2006; Wendland et al., 2006).

Whereas most of the work on gene-environment (GxE) interplay following the seminal work by Caspi et al. (2003) has focused on the interaction of 5-HTTLPR with major life events (e.g., Klauke, Deckert, Reif, Pauli, Zwanzger et al., 2011), impact of recent stressors in terms of DH or recent chronic stress are understudied, particularly with regard to anxiety. One of few studies showed that among college students S allele carriers experienced more anxious mood on days with more stress than L allele carriers (Gunthert et al., 2007). In contrary, Ming et al. (2015) found that adolescent L allele carriers exhibited more anxiety symptoms related to stressful life events, e.g. school or friendship problems. Apart from methodological differences, the heterogeneity of the findings may to some extent be explained by positive factors countering a GxE risk profile.

Recently, an extended approach to the GxE interaction model to include coping (C) characteristics in GxExC model (c.f.,

Schiele, Herzog, Kollert, Schartner et al., 2020) has highlighted the necessity of considering both advantageous and disadvantageous influences in shaping anxiety risk. High levels of general self-efficacy were observed to buffer an otherwise increased vulnerability to anxiety as conferred by the interaction of childhood adversity and 5-HTTLPR genotype (Schiele, Ziegler et al., 2016). These results are in line with the "differential susceptibility hypothesis" (Belsky et al., 2009), which postulates that genes are neither entirely favorable nor unfavorable, but rather drive sensitivity to environmental influences as a whole.

Resilience, the process of sustaining or strengthening physiological or behavioral stability in response to stressors, may be one prominent advantageous candidate. Hjemdal, Vogel, Solem, Hagen, and Stiles (2011) showed that lower resilience was associated with higher levels of anxiety in an adolescent sample. In line, higher levels of resilience were associated with lower anxiety symptom levels in an adolescent sample (Skrove et al., 2013) and in a student sample (Haddadi & Besharat, 2010).

To date, the integration of resilience in the context of GxE models in respect to anxiety is still rare. The only study known investigating a GxE interaction while considering moderating effects of resilience-increasing factors regarding anxiety was performed in adults (Schiele, Ziegler et al., 2016), whereas to the best of our knowledge no study focused on adolescence, i.e. the developmental period when anxiety often becomes pathological (Beesdo-Baum & Knappe, 2012). As adolescence is characterized by major psychophysiological and social changes (Sawyer et al., 2012), studying the interplay of genes, stressors, and resilience factors appears crucial to improve our understanding of emerging pathological anxiety during adolescence. Therefore, the aim of the current study is to investigate the interactive effect of 5-HTTLPR and recent stress on anxiety in a general population sample of adolescents by addressing the question whether resilience might buffer a GxE risk factor constellation.

2 | METHODS

2.1 | Sample and Procedures

The Behavior and Mind Health (BeMIND) study is a cohort study of a general population sample of adolescents and young adults from Dresden, Germany, examining developmental trajectories of mental and behavioral disorders. The current study uses baseline data (N = 1180, AAPOR formula 1 response rate: 21.7%, cooperation rate: 43.4%; AAPOR, 2016). The study protocol and its amendments were accepted by the ethics committee of the Technische Universität Dresden (TUD: EK381102014). Details on sampling and methods of the BeMIND study are provided elsewhere (Beesdo-Baum, Voss et al., 2020).

In short, an age- and sex-stratified random sample of 14–21 years old was drawn from the population registry in 2015, followed by a written invitation letter sent by the study team with a maximum of two reminder letters. All noninstitutionalized individuals aged

14–21 residing in a household in Dresden during the field period with sufficient German language skills were eligible to participate. Of 6321 invited subjects, 14.1% were found to be ineligible, mostly due to the fact that they were not residing under the provided address. Of the remaining 5428 individuals, 1180 participated in the study assessments which were conducted between November 2015 and December 2016 at the study center at the Technische Universität Dresden. Participation was higher among females and among those with higher education. Total 42.8% of invited individuals did not answer the invitation letters and the nonresponder questionnaire. Lack of time and interest were the most common given reasons for nonparticipation (Beesdo-Baum, Voss et al., 2020).

Participants underwent a standardized clinical-diagnostic assessment, an experimental assessment approximately one week later, and an ecological momentary assessment (EMA) as well as an online questionnaire assessment in between these two personal appointments. Biological or physiological data were collected during the EMA period (saliva, heart rate) and at the second personal appointment (blood/buccal, hair, anthropometric measures, and blood pressure). All participants provided written informed consent or assent and written informed consent of all legal guardians were gathered of those aged under 18 years. Participants received 50 Euro as incentive.

Genotypes from blood samples are available for 939 participants and from buccal swabs for 140 participants, respectively, resulting in a total of 1079 adolescents and young adults with available genetic data (91.4%). Participants were excluded from the current analysis if they had no caucasian descent by first generation ($N = 115$) or if information about the descent were not available ($N = 35$), resulting in an analysis sample of 942 of originally 1180 participants (79.8%).

2.2 | Assessments

Participants completed a range of self-report questionnaires, among those measures assessing symptoms of anxiety as well as measures on recent stress and resilience.

The Patient Reported Outcome Measurement Information System Adult Anxiety Version 1.0 Short Form (PROMIS-ANX; Pilkonis et al., 2011) is an eight-item scale assessing the frequency of anxiety symptoms over the past 7 days. Each item is rated on a five-point scale resulting in a score from 8 to 40.

Three different measures assessed recent stress. The DH scale (Perkonigg & Wittchen, 1995) is a self-report questionnaire based on the social interview schedule (Faltermaier et al., 1985), which assesses irritating and/or frustrating demands of everyday life during the past 2 weeks using 15 four-point scaled items.

The perceived stress scale (PSS-4; Cohen & Williamson, 1988) is a four-item version of the PSS (Cohen, Kamarck, & Mermelstein, 1983), assessing "the degree to which situations in one's life are appraised as stressful" (Cohen et al., 1983; p.387). The items are linked to a sole latent trait measuring global stress levels, the degree to which life has been experienced as unpredictable, uncontrollable and overloaded in the past month. Each item is measured on a five-point Likert scale (Stächele & Volz, 2013).

The 12-item screening scale of the trier inventory for the assessment of chronic stress (TICS-SSCS; Schulz et al., 2004) is a standardized questionaire measuring chronic overall self-perceived stress in the last three months on a five-point Likert scale, containing 12 of the original 57 items of the TICS (Schulz et al., 2004).

The Connor–Davidson resilience scale (CD-RISC; Connor & Davidson, 2003) is a 10-item self-rating scale that measures the ability to cope with adversity. Items are rated on a five-point Likert scale resulting in total sum-score from 0 to 40.

Higher values indicate higher levels of DH, perceived stress, chronic stress, or resilience on the respective questionnaires.

Diagnostic status was assessed with an updated version of the Munich composite international diagnostic interview (Wittchen & Pfister, 1997) providing lifetime and 12-month diagnoses of a range of mental disorders according to *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (Hoyer et al., 2020). The fully standardized computer-assisted personal interviews were conducted face-to-face by trained clinical interviewers.

Sociodemographic information containing age, sex, education, and subjective social status were assessed during the interview and Caucasian descent during the online questionnaire assessment.

2.3 | Genotyping

Two 9 ml EDTA blood samples were collected by venipuncture using the vacuum method at baseline. Blood samples were stored immediately at −80°C. Whenever participants did not provide consent or assent to draw blood, they were asked to provide a buccal sample instead. *5-HTT*LPR and the functionally related single-nucleotide polymorphism rs25531 were genotyped according to published protocols. In short, extracted DNA was amplified by polymerase chain reaction (60 s at 94°C, 60 s at 64°C, and 120 s at 72°C for 35 cycles) with the following oligonucleotide primers F: 5′-TGCCGCTCTGAATGCCAGCAC-3′ and R: 5′-GGGA TTCTGGTGCCACCTAGACG-3′. PCR products were digested with MspI at 37°C overnight, separated for 3.5 h on 3% ethidium bromide containing agarose gel and visualized by ultraviolet light (ChemiDoc UV chamber; BioRad) (for details see Schiele, Ziegler et al., 2016). Genotypes of 1079 adolescents were determined by two independent, blinded investigators. Hardy–Weinberg criteria were fulfilled for *5-HTT*LPR genotype distribution (SS = 131, SL = 444, LL = 367, $p = .859$) as well as for the triallelic model ($L_AL_A = 302$, $L_GL_A/SL_A = 458$, $L_GL_G/SL_G/SS = 182$, $p = .723$).

2.4 | Statistical analysis

To improve representativeness regarding sex and age, sample weights were applied to make sure that the sample distribution of sex and age is equal to the one of the target population of the 14–21

years old people living in Dresden (for details see Beesdo-Baum, Voss et al., 2020). The sample was stratified for genotype group according to functionality (Hu et al., 2006; Wendland et al., 2006) and previous publications (Baffa et al., 2010; Baune et al., 2008; Klauke, Deckert, Reif, Pauli, Zwanzger et al., 2011; Schiele, Ziegler et al., 2016; Schiele, Herzog, Kollert, Böhnlein et al., 2020; Wang et al., 2011; Wendland et al., 2006) resulting in a high-expression group (L_AL_A carriers; $N = 302$) and a low-expression group (SS, SL_G, SL_A, L_GL_A, L_GL_G carriers; $N = 640$). A group comparison between the low-and high-expression group as well as between the excluded participants from the total sample and the analysis sample was conducted regarding sociodemographic characteristics including age (t test), sex distribution, education, social class, lifetime psycho-pathology (survey design-based F test; Rao & Scott, 1984) as well as the predictor variables (DH, PSS-4, TICS-SSCS, CD-RISC) and the outcome variable (PROMIS-ANX; t test). Pairwise weighted correlations between the different stress indicators were calculated (using Stata command corr_svy; Winter, 2001), corresponding p values were taken from simple linear regressions between two stress in-dicators. Mean values were calculated for the stress scales DH, PSS-4, and SSCS-TICS and for CD-RISC. For regression analyses, the PROMIS-ANX score representing the outcome (dependent) variable was linearly rescaled to a range between zero (represents raw score of 8) and one (represents raw score of 40). Then, fractional response regression models (Papke & Wooldridge, 1996) were used, which are a viable tool if outcome data is skewed and many values occur at the lowest or highest possible outcome value. Also, the answer type of the PROMIS items suggests modeling the PROMIS score as a specific amount from a predefined maximum, that is, a fraction. Fractional logistic regression were used to determine the effects of 5-HTT genotype, stress scale (i.e., DH, STICS-SSCS, or PSS-4), and resilience (CD-RISC), as well as their interaction, on anxiety (PROMIS-ANX). The analyses were adjusted for age and sex and in a second step the presence of any lifetime mental disorder was included as covariate. Since regression coefficients of fractional response models are hard to interpret, plots of predictive margins for PROMIS-ANX from the estimated fractional response models are presented. For illustration purpose, participants above the CD-RISC sample mean were defined as "high-resilience group" and partici-pants below the CD-RISC sample mean as "low-resilience group." Final N's in the analysis vary due to missing values in the questionnaire assessments (ranging from 18–100).

3 | RESULTS

3.1 | Descriptive statistics

Descriptive characteristics of the analysis sample are given in Table 1. Genotype groups did not differ significantly regarding age ($p = .492$), sex ($p = .559$), education ($p = .102$), social status ($p = .530$), stress (DH, $p = .792$; TICS-SSCS, $p = .104$; PSS-4, $p = .645$), resilience

(CD-RISC, $p = .893$), anxiety (PROMIS-ANX, $p = .105$), or any lifetime mental disorder ($p = .151$). When comparing participants excluded from the total sample for the analysis ($N = 238$) and the final analysis sample ($N = 942$), no differences could be discerned regarding age ($p = .723$), sex ($p = .559$), social status ($p = .214$), and stress (DH, $p = .204$; TICS-SSCS, $p = .057$; PSS-4, $p = .074$). With respect to edu-cation, the analysis sample consisted of more participants with higher education levels (77.6 %w vs. 72.0 %w, design-based $F(2.87, 3380.18) = 6.16$, $p < .001$) and less participants with any lifetime mental disorder (50.9 %w vs. 60.3 %w, design-based $F(1, 1179) = 5.84$, $p = .016$). Regarding anxiety (PROMIS-ANX), excluded participants reported significantly higher levels of anxiety ($\beta = -.027$, $t = -2.60$, $p = .009$, confidence interval [CI]: [−0.04, −0.01]), while with respect to CD-RISC, the analysis sample reported higher resilience ($\beta = .146$, $t = 2.33$, $p = .020$, CI [0.02, 0.27]).

3.2 | Intercorrelations between the stress scales

Considering the conceptual overlap, there were significant correla-tions between the stress scales: DH and SSCS-TICS $r(841) = .49$, $p < .001$; DH and PSS-4 $r(841) = .44$, $p < .001$; and SSCS-TICS and PSS-4 $r(863) = .67$, $p < .001$.

3.3 | Effect of 5-HTT genotype, recent stress, and resilience on anxiety

To present the results of the analyses precisely, beta coefficients of fractional logit models are reported. These beta coefficients are not to be confused with the usual beta coefficients from linear regression models. For interpretation, please see the figures depicting pre-dictive margins calculated from the fractional logit models.

3.4 | Genotype x daily hassles x resilience

Data was available for $N = 825$ participants. There was a significant main effect of 5-HTT genotype ($\beta = -3.03$, $t = -2.04$, $p = .042$, CI [−5.94, −0.12]) and resilience ($\beta = -1.27$, $t = -3.49$, $p = .001$, CI [−1.98, −0.56]) on anxiety. Regarding interactions, the two-way interactions of 5-HTT genotype x DH ($\beta = 1.64$, $t = 2.43$, $p = .015$, CI [0.31, 2.96]) and 5-HTT genotype x resilience ($\beta = 1.20$, $t = 2.32$, $p = .021$, CI [0.18, 2.22]) as well as the three-way-interaction of 5-HTT genotype x DH x resilience were significant. Decomposing the interaction revealed that resilience buffered the impact of DH on anxiety among L_AL_A carriers ($\beta = -.63$, $t = -2.55$, $p = .011$, CI [−1.12, −0.15], illustrated in Figure 1. After adjusting for any lifetime mental disorder all effects remained significant with a main effect of 5-HTT genotype ($\beta = -3.09$, $t = -2.06$, $p = .040$, CI [−6.03, −0.56]) and resilience ($\beta = -.1.29$, $t = -3.65$, $p < .001$, CI [−1.98, −0.59]), with two-way interactions of 5-HTT genotype x DH ($\beta = 1.67$, $t = 2.48$, $p = .013$, CI [0.35, 2.98]),

TABLE 1 Sample characteristics

| | 5-HTTLPR/rs25531 | | | | | | | | | | | |
| | Analysis sample | | | | $L_A L_A$ | | | | SS, $L_G L_A$, SL_A, $L_G L_G$, SL_G, SS | | | |
	N	M	SD	%w	N	M	SD	%w	N	M	SD	%w
Age (years)	942	17.92	2.33		302	18.00	2.29		640	17.88	2.36	
14–17	540			41.4	155			51.3	355			42.8
18–21	432			58.6	147			48.7	285			57.2
Sex	942				302				640			
Male	393			51.3	121			50.3	272			51.8
Female	549			48.7	181			49.7	368			48.2
Education	942				301				640			
Low	13			1.4	2			0.4	11			1.9
Middle	178			17.9	47			15.2	131			19.2
High	715			77.6	240			80.9	475			76.1
Other	36			3.1	13			3.5	23			2.9
Social class	933				299				634			
Lowest	19			2.6	4			1.8	15			3.0
Lower middle	103			13.3	36			14.4	67			12.8
Middle	581			61.5	177			58.5	404			63.0
Upper middle	225			22.1	79			24.6	146			20.8
Upper	5			0.6	3			0.8	2			0.5
Any lifetime mental disorder	942				302				640			
Yes	456			50.9	145			47.2	311			52.7
No	486			49.1	157			52.8	329			47.3
DH	841	1.77	0.46		270	1.77	0.49		571	1.76	0.44	
PSS-4	871	2.38	0.71		278	2.40	0.72		593	2.37	0.71	
TICS-SSCS	863	1.22	0.73		276	1.28	0.77		587	1.19	0.71	
CD-RISC	856	2.78	0.68		275	2.79	0.72		581	2.78	0.66	
PROMIS-ANX	924	0.07	0.10		298	0.08	0.11		626	0.06	0.09	

Note: Data were weighted to improve representativeness for sex and age, but frequencies are reported unweighted. %w, weighted percentages; 5-HTTLPR/rs25531 genotype groups (LALA vs. SS, LGLA, SLA, LGLG, SLG, SS).

Abbreviations: CD-RISC, Connor–Davidson resilience scale; PROMIS-ANX, Patient Reported Outcome Measurement Information System Adult Anxiety Version 1.0 Short Form; TICS-SSCS, screening scale of the trier inventory for the assessment of chronic stress.

5-HTT genotype x resilience (β = 1.25, t = 2.38, p = .017, CI [0.22, 2.27]) and resilience x DH (β = .37, t = 2.08, p = .038, CI [0.02, 0.72]), and the three-way-interaction of 5-HTT genotype x DH x resilience (β = −.65, t = −2.66, p = .008, CI [−1.13, −0.17]).

3.5 | Genotype x chronic stress x resilience

Data was available for N = 840 participants. There were significant main effects of 5-HTT genotype (β = −1.68, t = −2.10, p = .036, CI [−3.25, −0.11]) and resilience (β = −.80, t = −4.10, p < .001,

CI [−1.18, −0.41]) on anxiety. All two-way interactions were significant, 5-HTT genotype x TICS-SSCS (β = .95, t = 2.54, p = .011, CI [0.21, 1.68]), 5-HTT genotype x resilience (β = .62, t = 2.35, p = .019, CI [0.10, 1.14]) and chronic stress x resilience (β = .24, t = 2.36, p = .018, CI [0.04, 0.44]). The three-way interaction of 5-HTT genotype x chronic stress x resilience (β = −.35, t = −2.55, p = .011, CI [−0.62, −0.08]) was significant as well. Figure 2 shows that high resilience was able to buffer the impact of chronic stress as measured with TICS-SSCS on anxiety in $L_A L_A$ carriers. After adjusting for any lifetime mental disorder all effects remained significant with a main effect of 5-HTT genotype (β = −1.81, t = −2.22, p = .027, CI [−3.41, −0.21]), resilience (β = −.77,
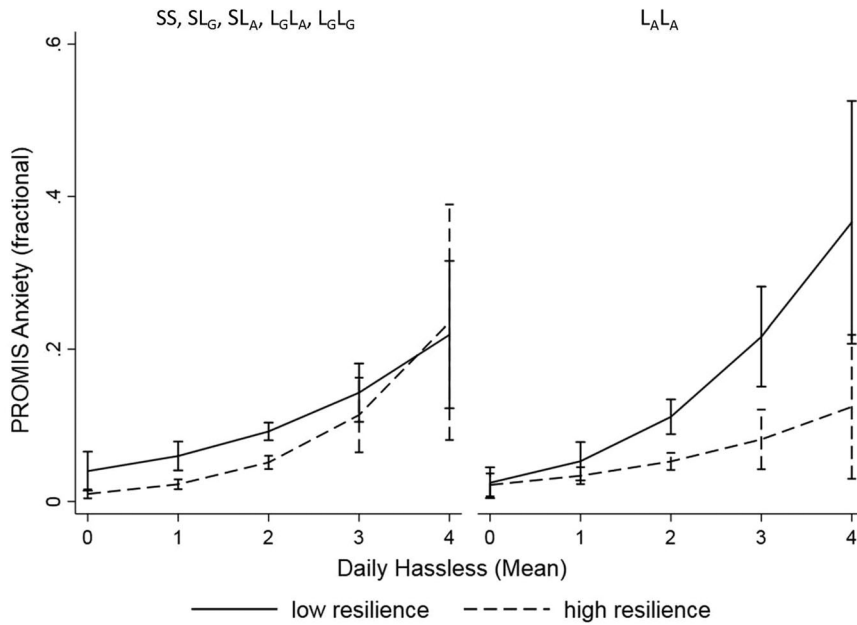
FIGURE 1 Effect of daily hassles on Patient Reported Outcome Measurement Information System Anxiety (PROMIS-ANX) as a function of *5-HTT*LPR/rs25531 and resilience. Illustrations of predictive margins with 95% confidence intervals (CIs) of fractional logistic regression analyses. *5-HTT*LPR/rs25531 genotype groups (L_AL_A vs. L_GL_G, SL_G, SS, L_GL_A, SL_A) and low versus high resilience measured by Connor–Davidson resilience scale (CD-RISC; cut-off = 2.73)

$t = -4.07$, $p < .001$, CI [−1.14, −0.40]), two-way interactions of *5-HTT* genotype x chronic stress ($\beta = 1.01$, $t = 2.62$, $p = .009$, CI [0.25, 1.78]), *5-HTT* genotype x resilience ($\beta = .67$, $t = 2.49$, $p = .013$, CI [0.14, 1.20]) and resilience x chronic stress ($\beta = .24$, $t = 2.39$, $p = .017$, CI [0.04, 0.43]), and the three-way-interaction of *5-HTT* genotype x chronic stress x resilience ($\beta = -.37$, $t = -2.63$, $p = .009$, CI [−0.65, −0.09]).

3.6 | Genotype x perceived stress x resilience

Data was available for $N = 840$ participants. There was no significant main effect of *5-HTT* genotype ($\beta = -2.15$, $t = -1.70$, $p = .090$, CI [−4.64, 0.33]), but a significant main effect of resilience ($\beta = -.80$, $t = -3.28$, $p = .001$, CI [−1.27, −0.32]) on anxiety. No

significant interaction of *5-HTT* genotype x PSS-4 ($\beta = .76$, $t = 1.90$, $p = .057$, CI [−0.02, 1.54]) and no significant interaction of *5-HTT* genotype x resilience ($\beta = .72$, $t = 1.75$, $p = .081$, CI [−0.09, 1.53]) was found. The three-way interaction of *5-HTT* genotype x perceived stress x resilience was also not significant ($\beta = -.24$, $t = -1.76$, $p = .079$, CI [−0.51, 0.03]) as shown in Figure 3. After adjusting for any lifetime mental disorder no significant main effect of *5-HTT* genotype ($\beta = -2.39$, $t = -1.82$, $p = .068$, CI [−4.96, 0.18]), but a significant main effect of resilience ($\beta = -.79$, $t = -3.28$, $p = .001$, CI [−1.27, −0.32]), and a significant interaction of *5-HTT* genotype x perceived stress ($\beta = .83$, $t = 1.99$, $p = .047$, CI [.01, 1.65]) on anxiety were discerned. The three-way-interaction of *5-HTT* genotype x perceived stress x resilience ($\beta = -.26$, $t = -1.85$, $p = .065$, CI [−0.54, 0.02]) was not significant.
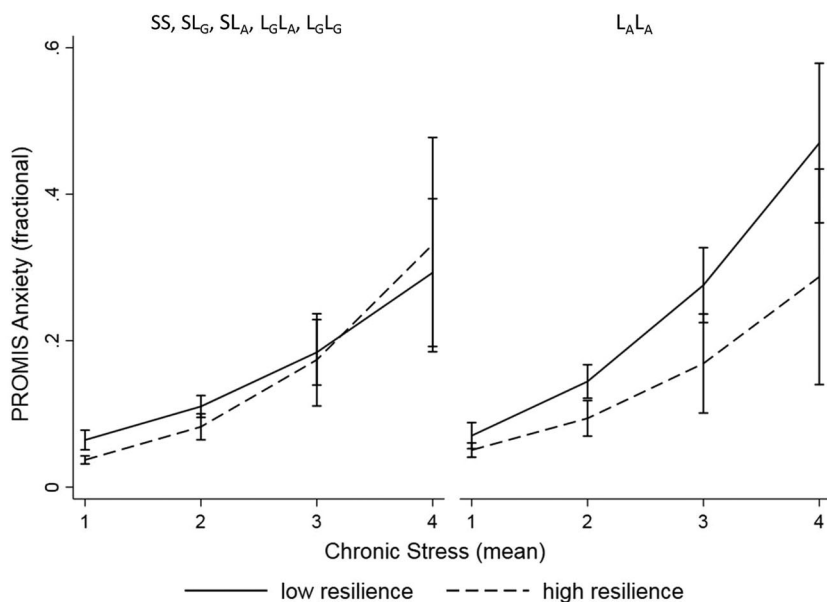

FIGURE 2 Effect of subjective chronic stress on PROMIS Anxiety as a function of *5-HTT*LPR/rs25531 and resilience. Illustrations of predictive margins with 95% CIs of fractional logistic regression analyses. *5-HTT*LPR/rs25531 genotype groups (L_AL_A vs. L_GL_G, SL_G, SS, L_GL_A, SL_A) and low versus high resilience measured by CD-RISC (cut-off = 2.73). CD-RISC, Connor–Davidson resilience scale; CI, confidence interval; PROMIS, Patient Reported Outcome Measurement Information System

FIGURE 3 Effect of perceived stress on PROMIS Anxiety as a function of 5-HTTLPR/rs25531 and resilience. Illustrations of predictive margins with 95% CIs of fractional logistic regression analyses. 5-HTTLPR/rs25531 genotype groups (L_AL_A vs. L_GL_G, SL_G, SS, L_GL_A, SL_A) and low versus high resilience measured by CD-RISC (cut-off = 2.73). CD-RISC, Connor–Davidson resilience scale; CI, confidence interval; PROMIS, Patient Reported Outcome Measurement Information System
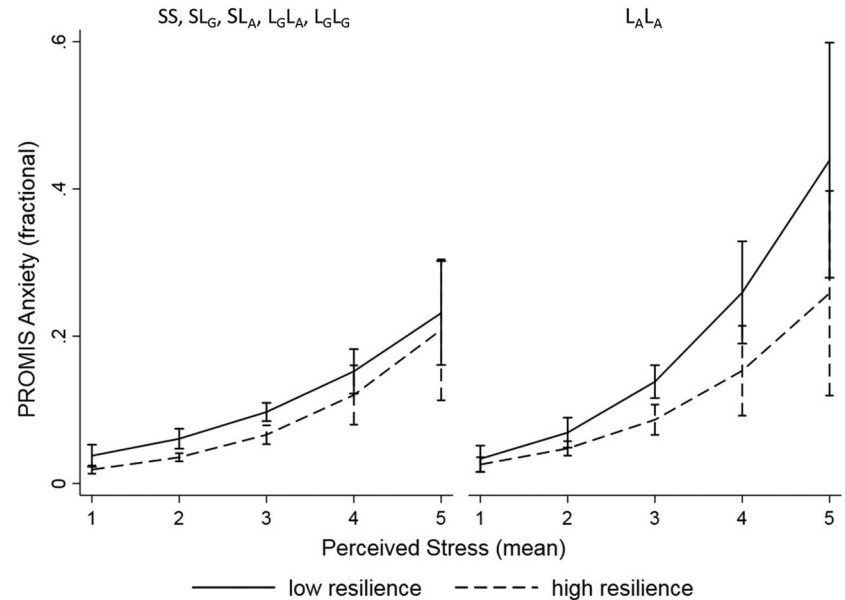
4 | DISCUSSION

The aim of the current study was to examine the interactive effect of SCL6A4 genotype and recent stress on anxiety, by addressing the question whether resilience is able to buffer a GxE risk constellation in adolescents and young adults. Resilience was found to interact with recent stress, such as DH and recent chronic stress, and 5-HTTLPR regarding anxiety. Specifically, adolescents carrying the more active L_AL_A genotype reported consistently higher levels of anxiety when they experienced more DH or recent chronic stress and had low levels of resilience. When the resilience scores were high, L_AL_A carriers reported the lowest anxiety scores in spite of DH or recent chronic stress.

These findings are in line with a study reporting that adolescent carriers of the L allele exhibited more anxiety symptoms related to stressful life events, for example, school or friendship problems (Ming et al., 2015). Contrary, Gunthert et al. (2007) found that the S allele rather than the L allele modified the effect of current daily stress on anxious mood in college students. These contradictory findings regarding the allelic direction of association may, however, be partly due to moderating positive influence as highlighted by the present study (c.f., Klauke, Deckert, Reif, Pauli, Zwanzger et al., 2011; Schiele, Ziegler et al., 2016). Consequently, a previous study has focused on the positive end that may counteract the deleterious impact of life stressors and serve to compensate for a biological risk profile. The interplay between anxiety traits and childhood trauma was found to be moderated by self-efficacy in 5-HTTLPR/rs25531 L_AL_A genotype carriers. Carriers of the L_AL_A genotype scored highest on different measures of anxiety if they had experienced childhood maltreatment, but only when general self-efficacy was low. When general self-efficacy was high, L_AL_A carriers had the lowest anxiety levels despite childhood adversities (Schiele, Ziegler et al., 2016). The present study showed for the first time, that beside major stressors minor stressors like DH and recent chronic stress interact with 5-HTTLPR in a GxE risk factor constellation and can be buffered by resilience.

The findings of the present study support the "differential susceptibility hypothesis" (Belsky et al., 2009) stating that a given genotype (in this case L_AL_A) does not transfer vulnerability for anxiety per se, but is rather subject to positive as well as negative environmental influences. In relation to this study, L_AL_A carriers reported higher levels of anxiety in the presence of more DH or recent chronic stress when resilience was low but at the same time, L_AL_A carriers were able to counterbalance the negative effects of DH and recent chronic stress if their resilience was high. This illustrates that the L_AL_A genotype might rather constitute a "plasticity" factor than a "risk" genotype. The present extended, three-dimensional GxE approach incorporating the buffering effect of resilience-increasing environmental factors (c.f., Schiele, Herzog, Kollert, Schartner et al., 2020) might thus aid in reconciliating incongruent or non-replicable two-dimensional 5-HTT GxE studies (e.g., Border et al., 2019).

Moreover, contradictory findings concerning the allelic direction of 5-HTTLPR or non-replicability could result from differences in investigated samples. Studies have shown that the effect of environmental influence on phenotypic variation may depend on the developmental stage. Consistently, Zalsman et al. (2015) showed that stress in pre-pubertal or adolescent developmental phases differentially influence structural integrity of specific brain regions as well as emotion regulation in a rat model depending on genetic background.

Concerning the nonsignificant results of PSS-4 compared with DH and TICS-SSCS might be due to differences in the constructs assessed by the scales. Whereas TICS-SSCS captures chronic psychosocial stress (Petrowski et al., 2012; p.43), independently of specific situations or domains in everyday life, the PSS-4 rather measures the degree to which life has been praised as stressful.

Some limitations need to be considered regarding the present findings. Only cross-sectional data was used for the current analysis. Although anxiety was assessed temporally after the stress-exposures, given the close proximity (few days) between the assessment of anxiety and stress or hassles, overlap in time scales cannot be excluded in most cases. Thus, future studies should focus on clearly prospective-longitudinal research. In addition, genetic data or information on the Caucasian descent for some participants with genetic data was not available, reducing the overall available sample size for the current analysis. Finally, the total BeMIND sample is regional and characterized by a relatively high educational level restricting the generalization of the results to adolescents and young adults with other backgrounds.

Despite the limitations, our findings from a general population sample of adolescents and young adults emphasize the focus on DH and recent chronic stress in a GxE framework, which is in line with other studies also pointing towards the influence of DH on anxiety (Barrett & Heubeck, 2000; D'Angelo & Wierzbicki, 2003). In further studies, additional factors like epigenetic processes modulating gene function and temporally dynamic processes susceptible to environmental influences, such as daily life stress respect to anxiety (for a review see Gottschalk et al., 2020; Schiele & Domschke, 2018) in particular *SLC6A4* DNA methylation (Domschke et al., 2014; Duman & Canli, 2015; Kang et al., 2013; Roberts et al., 2014; Schiele, Kollert et al., 2019) have to be considered.

In conclusion, the present study suggests that minor stressors, such as DH or recent chronic stress, in adolescent and young adults with low levels of resilience are in concert with higher levels of anxiety in carriers of the more active $L_A L_A$ genotype. However higher levels of resilience buffer against the background of a genetic risk constellation resulting in the lowest level of anxiety in $L_A L_A$ carriers with high levels of resilience despite the existence of DH or recent chronic stress. The integration of resilience in the GxE model regarding anxiety is broadening the scope by the incorporation of a "differential susceptibility" and "plasticity" framework. The consideration of positive and negative environmental influences along with genetic make-up carries potential for further research and clinical practice. Testing the effects of early interventions to build resilience targeted at individuals with higher DH or chronic stress and genetic susceptibility may be useful to prevent an escalation of distress and associated unfavorable health outcomes.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

The authors would like to thank the adolescents and young adults, as well as their families, for their participation in the BeMIND Study and for providing their valuable time to complete the study assessments. The authors also would like to thank the many study assistants for their excellent work in data collection and technical assistance.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Theresa M. Ollmann https://orcid.org/0000-0003-0290-6044
Katja Beesdo-Baum https://orcid.org/0000-0002-9687-5527

REFERENCES

APA. (2013). *Diagnostic and statistical manual of mental disorders* (5 ed.). American Psychiatric Association.

AAPOR. (2016). *Standard definitions: Final dispositions of case codes and outcome rates for surveys* (9th ed.): The American Association for Public Opinion Research.

Asselmann, E., Wittchen, H. U., Lieb, R., & Beesdo-Baum, K. (2017). A 10-year prospective-longitudinal study of daily hassles and incident psychopathology among adolescents and young adults: Interactions with gender, perceived coping efficacy, and negative life events. *Social Psychiatry and Psychiatric Epidemiology*, 52(11), 1353–1362.

Baffa, A., Hohoff, C., Baune, B. T., Mueller-Tidow, C., Tidow, N., Freitag, C., & Domschke, K. (2010). Norepinephrine and serotonin transporter genes: Impact on treatment response in depression. *Neuropsychobiology*, 62(2), 121–131. https://doi.org/10.1159/000317285

Barrett, S., & Heubeck, B. G. (2000). Relationships between school hassles and uplifts and anxiety and conduct problems in Grades 3 and 4. *Journal of Applied Developmental Psychology*, 21(5), 537–554.

Baune, B. T., Hohoff, C., Mortensen, L. S., Deckert, J., Arolt, V., & Domschke, K. (2008). Serotonin transporter polymorphism (5-HTTLPR) association with melancholic depression: A female specific effect? *Depression and Anxiety*, 25(11), 920–925. https://doi.org/10.1002/da.20433

Beesdo-Baum, K., & Knappe, S. (2012). Developmental epidemiology of anxiety disorders. *Child and Adolescent Psychiatric Clinics*, 21(3), 457–478.

Beesdo-Baum, K., Voss, C., Venz, J., Hoyer, J., Berwanger, J., Kische, H., & Pieper, L. (2020). The Behavior and Mind Health (BeMIND) study: Methods, design and baseline sample characteristics of a cohort study among adolescents and young adults. *International Journal of Methods in Psychiatric Research*, 29(1), e1804. https://doi.org/10.1002/mpr.1804

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., & Williams, R. (2009). Vulnerability genes or plasticity genes? *Molecular Psychiatry*, 14(8), 746–754. https://doi.org/10.1038/mp.2009.44

Border, R., Johnson, E. C., Evans, L. M., Smolen, A., Berley, N., Sullivan, P. F., & Keller, M. C. (2019). No support for historical candidate gene or candidate gene-by-interaction hypotheses for major depression across multiple large samples. *American Journal of Psychiatry*, 176(5), 376–387.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., & Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, *301*(5631), 386–389. https://doi.org/10.1126/science.1083968

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A gobal measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385–396. https://doi.org/10.2307/2136404

Cohen, S., & Williamson, G. (1988). Perceived stress in a probability sample of the US In: Spacapam S, Oskamp S, eds. The social psychology of health: Claremont Symposium on Applied Social Psychology: Sage.

Connor, K. M., & Davidson, J. R. (2003). Development of a new resilience scale: The Connor–Davidson resilience scale (CD-RISC). *Depression and Anxiety*, *18*(2), 76–82.

D'Angelo, B., & Wierzbicki, M. (2003). Relations of daily hassles with both anxious and depressed mood in students. *Psychological Reports*, *92*(2), 416–418.

Domschke, K., Tidow, N., Schwarte, K., Deckert, J., Lesch, K.-P., Arolt, V., Zwanzger, P., & Baune, B. T. (2014). Serotonin transporter gene hypomethylation predicts impaired antidepressant treatment response. *International Journal of Neuropsychopharmacology*, *17*(8), 1167–1176. https://doi.org/10.1017/s146114571400039x

Duman, E. A., & Canli, T. (2015). Influence of life stress, 5-HTTLPR genotype, and SLC6A4 methylation on gene expression and stress response in healthy Caucasian males. *Biology of Mood & Anxiety Disorders*, *5*, 5. https://doi.org/10.1186/s13587-015-0017-x

Faltermaier, T., Wittchen, H. U., Ellmann, R., & Lassle, R. (1985). The social interview schedule (SIS)-Content, structure, and reliability. *Social Psychiatry*, *20*(3), 115–124. https://doi.org/10.1007/bf00583577

Gottschalk, M. G., Domschke, K., & Schiele, M. A. (2020). Epigenetics underlying susceptibility and resilience relating to daily life stress, work stress, and socioeconomic status. *Frontiers in Psychiatry*, *11*, 163. https://doi.org/10.3389/fpsyt.2020.00163

Gunthert, K. C., Conner, T. S., Armeli, S., Tennen, H., Covault, J., & Kranzler, H. R. (2007). Serotonin transporter gene polymorphism (5-HTTLPR) and anxiety reactivity in daily life: A daily process approach to gene-environment interaction. *Psychosomatic Medicine*, *69*(8), 762–768. https://doi.org/10.1097/PSY.0b013e318157ad42

Haddadi, P., & Besharat, M. A. (2010). Resilience, vulnerability and mental health. *Procedia-Social and Behavioral Sciences*, *5*, 639–642.

Hjemdal, O., Vogel, P. A., Solem, S., Hagen, K., & Stiles, T. C. (2011). The relationship between resilience and levels of anxiety, depression, and obsessive–compulsive symptoms in adolescents. *Clinical Psychology & Psychotherapy*, *18*(4), 314–321.

Hoyer, J., Voss, C., Strehle, J., Venz, J., Pieper, L., Wittchen, H.-U., & Beesdo-Baum, K. (2020). Test-retest reliability of the computer-assisted DIA-X-5 interview for mental disorders. *BMC Psychiatry*, *20*, 1–16.

Hu, X. Z., Lipsky, R. H., Zhu, G. S., Akhtar, L. A., Taubman, J., Greenberg, B. D., Arnold, P. D., Richter, M. A., Kennedy, J. L, Murphy, D. L., & Goldman, D. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *American Journal of Human Genetics*, *78*(5), 815–826. https://doi.org/10.1086/503850

Kang, H.-J., Kim, J.-M., Stewart, R., Kim, S.-Y., Bae, K.-Y., Kim, S.-W., Shin, I.-S, Shin, M.-G, & Yoon, J.-S (2013). Association of SLC6A4 methylation with early adversity, characteristics and outcomes in depression. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, *44*, 23–28. https://doi.org/10.1016/j.pnpbp.2013.01.006

Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. S. (1981). Comparison of 2 modes odf stress measurement daily hassles and uplift vs. major life events. *Journal of Behavioral Medicine*, *4*(1), 1–40. https://doi.org/10.1007/bf00844845

Klauke, B., Deckert, J., Reif, A., Pauli, P., & Domschke, K. (2010). Life events in panic disorder—An update on "candidate stressors". *Depression and Anxiety*, *27*(8), 716–730. https://doi.org/10.1002/da.20667

Klauke, B., Deckert, J., Reif, A., Pauli, P., Zwanzger, P., Baumann, C., & Domschke, K. (2011). Serotonin transporter gene and childhood trauma—A G*E effect on anxiety sensitivity. *Depression and Anxiety*, *28*(12), 1048–1057. https://doi.org/10.1002/da.20840

Lazarus, R. S. (1986). Puzzles in the study of daily hassles, *Development as action in context* (pp. 39–53). Springer.

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Müller, C. R., Hamer, D. H., & Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*(5292), 1527–1531. https://doi.org/10.1126/science.274.5292.1527

Lesch, K. P., Zeng, Y., Reif, A., & Gutknecht, L. (2003). Anxiety-related traits in mice with modified genes of the serotonergic pathway. *European Journal of Pharmacology*, *480*(1-3), 185–204. https://doi.org/10.1016/j.ejphar.2003.08.106

Ming, Q., Zhang, Y., Yi, J., Wang, X., Zhu, X., & Yao, S. (2015). Serotonin transporter gene polymorphism (5-HTTLPR) L allele interacts with stress to increase anxiety symptoms in Chinese adolescents: a multiwave longitudinal study. *BMC Psychiatry*, *15*(1), 248.

Papke, L. E., & Wooldridge, J. M. (1996). Econometric methods for fractional response variables with an application to 401(k) plan participation rates. *Journal of Applied Econometrics*, *11*(6), 619–632. https://doi.org/10.1002/(Sici)1099-1255(199611)11:6<619::Aid-Jae418>3.0.Co;2-1

Perkonigg, A., & Wittchen, H. (1995). *The daily-hassles scale: Research version*. Max-Planck-Institute of Psychiatry.

Petrowski, K., Paul, S., Albani, C., & Brähler, E. (2012). Factor structure and psychometric properties of the trier inventory for chronic stress (TICS) in a representative German sample. *BMC Medical Research Methodology*, *12*(1), 42.

Pilkonis, P. A., Choi, S. W., Reise, S. P., Stover, A. M., Riley, W. T., & Cella, D., PROMIS Cooperative Group. (2011). Item banks for measuring emotional distress from the Patient-Reported Outcomes Measurement Information System (PROMIS®): Depression, anxiety, and anger. *Assessment*, *18*(3), 263–283.

Rao, J. N., & Scott, A. J. (1984). On chi-squared tests for multiway contingency tables with cell proportions estimated from survey data. *The Annals of statistics*, *12*, 46–60.

Roberts, S. R., Lester, K. J., Hudson, J. L., Rapee, R. M., Creswell, C., Cooper, P. J., Thirlwall, K. J., Coleman, J. R. I, Breen, G., Wong, C. C. Y., & Eley, T. C. (2014). Serotonin tranporter methylation and response to cognitive behaviour therapy in children with anxiety disorders. *Translational Psychiatry*, *4*, 4–e444. https://doi.org/10.1038/tp.2014.83

Sawyer, S. M., Afifi, R. A., Bearinger, L. H., Blakemore, S.-J., Dick, B., Ezeh, A. C., & Patton, G. C. (2012). Adolescence: A foundation for future health. *The Lancet*, *379*(9826), 1630–1640. https://doi.org/10.1016/S0140-6736(12)60072-5

Schiele, M. A., & Domschke, K. (2018). Epigenetics at the crossroads between genes, environment and resilience in anxiety disorders. *Genes, Brain and Behavior*, *17*(3), e12423. https://doi.org/10.1111/gbb.12423

Schiele, M. A., Herzog, K., Kollert, L., Böhnlein, J., Repple, J., Rosenkranz, K., Leehr, E. J., Zeigler, C., Lueken, U., Dannlowski, U., Pauli, P., Arolt, V., Zwanzger, P., Deckert, J., Erfurth, A., & Domschke, K. (2020). Affective temperaments (TEMPS-A) in panic disorder and healthy probands: Genetic modulation by 5-HTT variation. *The World Journal of Biological Psychiatry*, 1–7.

Schiele, M. A., Herzog, K., Kollert, L., Schartner, C., Leehr, E. J., Böhnlein, J., Repple, J., Rosenkranz, K., Lonsdorf, T. B., Dannlowski, U., Zwanzger, P., Reif, A., Pauli, P., Deckert, J., & Domschke, K. (2020). Extending the vulnerability–stress model of mental disorders: Three-dimensional NPSR1× environment × coping interaction study in anxiety. *The British Journal of Psychiatry*, 1–6.

Schiele, M. A., Kollert, L., Lesch, K. P., Arolt, V., Zwanzger, P., Deckert, J., Zeigler, C., & Domschke, K. (2019). Hypermethylation of the serotonin

transporter gene promoter in panic disorder-Epigenetic imprint of comorbid depression? *European Neuropsychopharmacology, 29*(10), 1161–1167. https://doi.org/10.1016/j.euroneuro.2019.07.131

Schiele, M. A., Ziegler, C., Holitschke, K., Schartner, C., Schmidt, B., Weber, H., Reif, A., Romanos, M., Pauli, P., Zwanzger, P., Deckert, J., & Domschke, K. (2016). Influence of 5-HTT variation, childhood trauma and self-efficacy on anxiety traits: A gene-environment-coping interaction study. *J Neural Transm, 123*(8), 895–904. https://doi.org/10.1007/s00702-016-1564-z

Schulz, P., Schlotz, W., & Becker, P. (2004). *Trierer inventar zum chronischen stress: TICS.* Hogrefe.

Skrove, M., Romundstad, P., & Indredavik, M. S. (2013). Resilience, lifestyle and symptoms of anxiety and depression in adolescence: The Young-HUNT study. *Social Psychiatry and Psychiatric Epidemiology, 48*(3), 407–416.

Stächele, T., & Volz, H.-P. (2013). Taschenatlas stress: Aesopus-Verlag.

Wang, Z., Baker, D. G., Harrer, J., Hamner, M., Price, M., & Amstadter, A. (2011). The relationship between combat-related posttraumatic stress disorder and the 5-HTTLPR/rs25531 polymorphism. *Depression and Anxiety, 28*(12), 1067–1073.

Wendland, J. R., Martin, B. J., Kruse, M. R., Lesch, K. P., & Murphy, D. L. (2006). Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular Psychiatry, 11*(3), 224–226. https://doi.org/10.1038/sj.mp.4001789

Winter, N. (2001). CORR_SVY: Stata module to compute correlation tables for survey data.

Wittchen, H. U., & Pfister, H. (1997). *DIA-X interview.* Swets & Zeitlinger.

Zalsman, G., Gutman, A., Shbiro, L., Rosenan, R., Mann, J. J., & Weller, A. (2015). Genetic vulnerability, timing of short-term stress and mood regulation: A rodent diffusion tensor imaging study. *European Neuropsychopharmacology, 25*(11), 2075–2085.