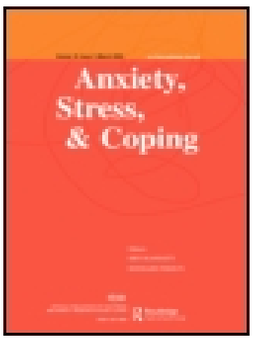

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Genetic variation in neuropeptide Y interacts with childhood trauma to influence anxiety sensitivity

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ABSTRACT

Background and objectives: Anxiety sensitivity (AS) refers to a fear of the negative implications of anxiety, and arises due to gene-environment interactions. We investigated whether genetic variation in two neuropeptides implicated in the stress response, neuropeptide Y (NPY) and pituitary adenylate cyclase-activating polypeptide receptor 1, interacted with childhood trauma (CT) to influence AS.

Design and methods: This cross-sectional study examined the CT x genetic variant effects on AS in 951 adolescents who self-identified as Xhosa or South African Colored (SAC) ethnicity.

Results: In Xhosa females, the *NPY* rs5573 A allele and rs3037354 deletion variant were associated with increased ($p = 0.035$) and decreased ($p = 0.034$) AS, respectively. The interaction of CT and the *NPY* rs5574 A allele increased AS in SAC female participants ($p = 0.043$). The rs3037354 deletion variant protected against AS with increased CT in SAC male participants ($p = 0.011$).

Conclusions: The *NPY* rs5574 A allele and rs3037354 deletion variant interact with CT to act as risk and protective factors, respectively, for AS in an ethnicity- and sex- differentiated manner. Our results reaffirm the role of NPY and gene-environment interactions in anxiety-related behaviors and reinforce the need for psychiatric genetics studies in diverse populations.

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Introduction

Anxiety disorders are the most prevalent psychiatric disorders and impact significantly on quality of life (Stein et al., 2017). Anxiety sensitivity (AS) refers to the fear of anxiety, including its negative effects on physical, social and psychological wellbeing (McLaughlin & Hatzenbuehler, 2009; Schmidt et al., 2008, 2010; Stewart et al., 1997). Previous research has identified AS as a transdiagnostic risk marker for the development of psychopathology. AS has been linked to symptoms of panic disorder, posttraumatic disorder, obsessive compulsive disorder and borderline personality disorder, as well as suicidal ideation and suicide risk (Bounoua et al., 2015; Calamari et al., 2008; Jurin & Biglbauer, 2018; Li & Zinbarg, 2007; Overstreet et al., 2018; Stanley et al., 2018). In more recent studies, higher baseline AS has been associated with clinical levels of internalizing problems at six months follow-up in fourth grade children, as well as elevated depression and anxiety symptoms at two year follow-up in adolescents (Hernandez Rodriguez et al., 2020; Qi et al., 2019). AS has

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an estimated heritability of 45%, with both heritable and environmental factors contributing to its etiology (M. B. Stein et al., 1999). Childhood trauma (CT) is one such environmental factor (Martin et al., 2014; McLaughlin & Hatzenbuehler, 2009). Accordingly, our own research has indicated that CT experience interacted with serotonin and hypothalamic–pituitary–adrenal (HPA) axis variants to influence AS (Hemmings et al., 2016; Womersley et al., 2018). We sought to expand our investigation to other genetic variants implicated in the stress response that may contribute to AS in the aforementioned sample.

Neuropeptide Y (NPY) is a neurotransmitter that is highly expressed in the hypothalamus, hippocampus and amygdala, brain regions involved in the regulation of emotion, fear learning and memory, threat assessment, and the stress response (Eaton et al., 2007). Its proposed role in behavioral resilience and coping is partly due to its effect on the HPA axis (Morgan et al., 2002; Sah et al., 2014; Yehuda et al., 2006). Stress-induced release of corticotrophin releasing factor (CRF) leads to the release of NPY, such that CRF initiates the stress response, and NPY is involved in compensatory mechanisms required to facilitate a return to homeostasis (Sajdyk et al., 2004). Data obtained via direct experimental manipulations in animal models strongly suggest that the influence of NPY on anxiety-related states can also be explained by its abundant expression in brain areas implicated in emotional processing and fear-related memory, which may be independent of HPA axis effects. For example, NPY in the basolateral amygdala was found to be proportional to stress resiliency (Sajdyk et al., 2008), while NPY receptor activation was associated with hypoactivity in the lateral habenula, an area of the brain implicated in the processing of aversive experiences (Cheon et al., 2019). Furthermore, stress-induced reductions in hippocampal NPY are associated with increased synaptic plasticity in this area, thereby potentiating aversive memory formation (Li et al., 2017). The relationship between emotional processing and NPY activity in these, and other, brain areas has been recently reviewed (Comeras et al., 2019).

In human psychiatric genetics studies, single nucleotide polymorphisms (SNPs) in the *NPY* gene have been shown to influence *NPY* expression (Zhang et al., 2012; Zhou et al., 2008), and these genetically-driven variations are in turn associated with behavior and psychological wellbeing. The rs3037354 two nucleotide in/del has also been associated with stress reactivity, with the deletion (D) variant linked to greater sympathetic response and NPY secretion following an environmental stressor (Zhang et al., 2012). The *NPY* rs16147 C allele has been associated with higher posttraumatic stress disorder (PTSD) symptom scores in military veterans (Watkins et al., 2017) and increased amygdala responses to threatening faces (Domschke et al., 2010). Furthermore, previous studies have found that the combination of the C allele and adverse childhood experiences is predictive of higher trait anxiety (Sommer et al., 2010) and increased amygdala responsiveness during an emotional face processing task (Opmeer et al., 2014). Zhou et al. (2008) found that a seven-SNP haplotype (rs3037354-rs17149106-rs16147-rs16139-rs5573-rs5574-rs16475), predictive of lower NPY expression, was inversely related to trait anxiety and positively associated with greater amygdala response to threatening faces. Combined, these data suggest that genetic variation in *NPY,* both alone and in combination with CT, may influence emotional processing and anxiety-related behavior.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is also expressed in the hypothalamus and limbic brain structures and regulates emotional processing, fear-related memory and HPA axis activity (Ramikie & Ressler, 2016). However, following binding to the PACAP G protein-coupled receptor (PACAP1R), it acts to increase CRF secretion with anxiogenic effects (Agarwal et al., 2005; Hashimoto et al., 2011; Ramikie & Ressler, 2016). Genetic variation in *ADCYAP1R1*, the gene encoding PACAP1R, has also been shown to affect receptor expression (Ressler et al., 2011). For example, C allele homozygosity for the rs2267735 SNP has been associated with PTSD diagnosis in trauma-exposed women (Ressler et al., 2011), as well as increased amygdala and hippocampal activation in response to threatening faces (Stevens et al., 2014). This SNP has also been shown to interact with environmental factors to influence psychopathology, with experience of CT predictive of post-traumatic symptom severity in women homozygous for the C allele (Uddin et al., 2013).

Although evidence suggests that both NPY and PACAP are involved in mediating risk and resilience to stress- and anxiety-related disorders, its role in AS, a recognized predictor of later psychopathology, has yet to be investigated. Therefore, we conducted an exploratory study to examine the role of genetic variation in *NPY* and *ADCYAP1R1* in AS in a cohort of South African adolescents with varying levels of CT.

Methods

Participants and study design

The results reported in this manuscript form part of a broader investigation into AS in South African adolescents (Hemmings et al., 2016; Martin et al., 2014; Womersley et al., 2018). Briefly, 1,149 participants were recruited from 29 secondary schools in Cape Town, South Africa, as part of a cross-sectional study on AS. DNA was collected from 985 of these individuals. The present sample comprised 951 participants from the two primary self-identified ethnicities i.e., "Black, Xhosa" ($n = 634$) or "South African Colored" (SAC) ($n = 317$). All participants completed the clinical measures and provided saliva samples for DNA analyses. The study was approved by Stellenbosch University's Health Research Ethics Committee (project number N10/11/370) and permission to access schools was granted by the Western Cape Education Department. All participants, as well as a parent or legal guardian, provided written informed consent to participate in the study.

Clinical measures

Anxiety sensitivity was assessed using the Childhood Anxiety Sensitivity Index (CASI), an 18-item questionnaire measuring fear of anxiety in children and adolescents (Silverman et al., 1991). The CASI uses a three-point scale to grade beliefs around the extent to which anxiety can produce negative effects on physical, social and psychological measures. Responses are rated from one to three i.e., "none" to "a lot", to yield scores ranging from 18–54 with higher scores indicative of greater AS. A Cronbach's alpha value of 0.81 indicated good internal consistency for the CASI in our sample (Martin et al., 2014). The tendency to react fearfully to stressors was assessed using the 20-item Trait scale of the State-Trait Anxiety Inventory (STAI-T) (Spielberger, 1973). Experience of depressive symptoms in the week preceding assessment was determined using the 20-item Center for Epidemiological Studies Depression Scale for Children (CES-DC) (Weissman et al., 1980). The frequency and severity of physical, sexual and emotional abuse, as well as emotional and physical neglect, experienced prior to twelve years of age was evaluated using the 28-item Childhood Trauma Questionnaire (CTQ) (Bernstein & Fink, 1998). Response options are coded on a five-point Likert scale with higher scores indicative of greater exposure to CT. The CTQ has 5 maltreatment scales generating scale scores (range of 5–25), as well as total trauma scores (range of 25–125). In this sample, the internal consistency for the total CTQ score was good ($\alpha = 0.86$) and ranged from $\alpha = 0.53$ for the physical neglect scale to $\alpha = 0.80$ for the sexual abuse scale (Martin et al., 2014). Resilience was assessed with the 25-item Connor-Davidson Resilience Scale (CD-RISC) (Connor & Davidson, 2003). Coping behaviors used to manage stress were gauged using the 54-item Adolescent Coping Orientation for Problem Experiences (A-COPE) (Patterson & McCubbin, 1987). Finally, the Alcohol Use Disorders Identification Test (AUDIT), a 10-item questionnaire, was used to screen for hazardous alcohol use in the past year (Babor et al., 2001).

Genotyping

Genotyping for the four targeted *NPY* SNPs (rs16147, rs3037354, rs5573 and rs5574) and the single *ADCYAP1R1* SNP (rs2267735) was performed at McGill University and Génome Québec Innovation Centre as previously described (Womersley et al., 2018). Briefly, DNA extraction using Prep-It L2P

reagent (DNA Genotek, Ontario, Canada) was performed on saliva collected in Oragene™ DNA self-collection kits (DNA Genotek, Ontario, Canada). Genotyping was subsequently performed using Sequenom® iPLEX® Gold Genotyping Technology, which employs matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry and polymerase chain reaction (PCR) (Millis, 2011; Perkel, 2008). Multiplex PCR using SNP-specific primers was used to amplify DNA. Following removal of excess nucleotides, a single-base extension reaction, and desalting, the mass of products obtained by spectrometry were analyzed using MassARRAY Typer Analyser software (Agena Bioscience, San Digeo, CA). Genotyping was successfully performed for rs16147 ($n = 872$), rs3037354 ($n = 919$), rs5573 ($n = 884$), rs5574 ($n = 923$) and rs2267735 ($n = 868$).

Statistical analysis

We used general linear models to assess the association of genetic variation (genotype, additive allelic and haplotype, respectively) on CASI score. Briefly, genotype effects were assessed using genotype, dominant and recessive minor allelic models, while the additive allelic variable reflected the number of minor alleles carried by each participant. Pairs of haplotypes were inferred per participant and the calculated posterior probability of each subject having each possible haplotype was used for haplotype analyses. The reported effect sizes reflect the difference in CASI scores between specific and reference genetic factors. The interaction between CT scores and the same genetic factors on CASI score was also assessed. The interaction effect sizes are illustrated by the differences in slopes on plots of CASI versus CTQ, between the specific and the reference genetic factors. Analyses were stratified according to sex and self-reported ethnicity. All analyses included age, CES-DC, CD-RISC, A-COPE, STAIT and AUDIT scores as covariates. Statistical analyses were done using R (R Core Team, 2018) and the *haplo.stats* (Sinwell & Schaid, 2016) and *effects* packages (Fox, 2003). Results were deemed significant at $p < 0.05$.

Results

Demographic and clinical characteristics of the study group

Descriptive statistics for this study group have been previously reported (Hemmings et al., 2016; Womersley et al., 2018) and are included in Table 1. Of the 951 adolescents included, the majority ($n = 634$, 66.7%) self-identified as "Black, Xhosa-speaking", hereafter referred to as "Xhosa". The remainder ($n = 317$, 33.3%) self-identified as belonging to the SAC population, a unique population with a complex five-way genetic admixture (Daya et al., 2013). Participants in each of these populations were predominantly female (59.1% of Xhosa and 59.3% of SAC participants). Xhosa participants reported greater AS, trait anxiety and CT as well as lower resilience. South African Colored participants scored higher on the AUDIT measure of harmful and hazardous drinking behavior. The two groups did not otherwise differ on age, mean depression scores and mean coping scores.

Genetic analysis

Single nucleotide polymorphism analyses

Minor allele frequencies were calculated per sex and population group and are reported in Table 2. All five SNPs were in Hardy-Weinberg equilibrium in both Xhosa and SAC groups ($p > 0.05$). Linear models examining the association between genetic variants and CASI score provided two significant effects in Xhosa female participants (Table 3 and Figure 1). Under additive allelic models each rs5573 A allele was associated with an increase of 0.89 (95% CI: 0.06–1.71, $p = 0.035$) in CASI score, and each additional rs3037354 D variant was associated with an estimated 0.90 (95% CI: 0.07–1.73, $p = 0.034$) point reduction in CASI score.

Table 1. Summary of demographic and clinical variables stratified by sex and population group.

		Xhosa			SAC		
Characteristic	Summary	Male ($n = 259$)	Female ($n = 375$)	All ($n = 634$)	Male ($n = 129$)	Female ($n = 188$)	All ($n = 317$)
Age in years	Mean ± SD	16.4 ± 2.0	16.4 ± 2.1	16.4 ± 2.1	15.8 ± 1.5	15.8 ± 1.7	15.8 ± 1.6
CASI total score *	Mean ± SD	34.9 ± 6.2	36.9 ± 6.2	36.1 ± 6.3	31.5 ± 6.3	35.8 ± 6.6	34.1 ± 6.8
STAI-T *	Mean ± SD	45.9 ± 6.6	47.3 ± 8.3	46.7 ± 7.6	42.1 ± 8.7	46.8 ± 9.4	44.8 ± 9.4
CES-DC	Mean ± SD	21.8 ± 10.3	23.9 ± 11.1	23.0 ± 10.8	20.0 ± 11.9	27.0 ± 12.4	24.1 ± 12.6
CTQ *	Median (range)	44 (25-94)	43 (25-90)	43 (25-94)	39 (25-85)	40 (25-96)	40 (25-96)
CD-RISC	Mean ± SD	57.0 ± 19.7	57.3 ± 18.9	57.2 ± 19.2	62.87 ± 18.8	64.7 ± 17.6	64.0 ± 18.1
A-COPE	Mean ± SD	166.6 ± 23.1	166.5 ± 20.3	166.6 ± 21.5	166.1 ± 22.4	168.3 ± 22.3	167.4 ± 22.4
AUDIT *	Median (range)	0 (0-28)	0 (0-29)	0 (0-29)	2 (0-27)	1 (0-32)	2 (0-32)

A-COPE = Adolescent Coping Orientation for Problem Experiences; AUDIT = Alcohol Use Disorders Identification Test; CASI = Childhood Anxiety Sensitivity Index; CD-RISC = Connor-Davidson Resilience Scale; CES-DC = Center for Epidemiological Studies Depression Scale for Children; CTQ = Childhood Trauma Questionnaire; SAC = South African Colored; SD = standard deviation; STAIT = State-Trait Anxiety Inventory. * Characteristics significantly different between Xhosa and South African Colored groups ($p < 0.05$). * Characteristics significantly different between Xhosa and SAC groups ($p < 0.05$).

Table 2. Minor Allele Frequencies and Hardy-Weinberg *P*-values for NPY and ADCYAP1R1 Variants Stratified by Sex and Population Group.

					Minor allele frequency					
					Xhosa			SAC		
Gene	SNP	SNP location	Intronic/ exonic	Minor allele (major allele)	Male	Female	HWE	Male	Female	HWE
NPY	rs16147	chr7:24283791	Intronic	C (T)	0.340	0.358	0.652	0.405	0.392	0.105
	rs3037354	chr7:24283307	Intronic	D (I)	0.349	0.343	0.723	0.318	0.347	1.000
	rs5573	chr7:24285390	Intronic	A (G)	0.333	0.352	1.000	0.384	0.371	0.314
	rs5574	chr7:24289514	Intronic	A (G)	0.300	0.327	0.309	0.285	0.294	0.888
ADCYAP1R1	rs2267735	chr7:31095890	Intronic	G (C)	0.257	0.268	1.000	0.428	0.371	0.708

SAC = South African Colored.

Table 3. *P*-values of Association Tests between CASI Scores and SNPs Stratified According to Sex and Population.

		Xhosa				SAC			
		Genotype model		Allelic model		Genotype model		Allelic model	
Gene	SNP	Male	Female	Male	Female	Male	Female	Male	Female
NPY	rs16147	0.749	0.293	0.608	0.138	0.206	0.425	0.632	0.300
	rs3037354	0.162	0.106	0.381	0.034 *	0.215	0.659	0.080	0.363
	rs5573	0.607	0.109	0.499	0.035 *	0.208	0.419	0.593	0.320
	rs5574	0.624	0.155	0.527	0.055	0.253	0.121	0.700	0.325
ADCYAP1R1	rs2267735	0.580	0.553	0.410	0.277	0.252	0.859	0.994	0.619

SAC = South African Colored. The *p* values for individual tests of association under genotype and allelic models are shown. All models were adjusted for age, as well as STAI-T, CES-DC, CTQ, CD-RISC, A-COPE and AUDIT test scores. * significant effects on CASI score ($p < 0.05$).

Interaction analyses indicated that the combination of the additive allelic form of the rs5574 SNP and CT significantly influenced AS in SAC female participants (Table 4 and Figure 2). For each A allele, a unit increase in CTQ score yields a 0.116 (95% CI: 0.004–0.228, $p = 0.043$) point increase in the CASI score. Interaction analyses also indicated a significant CTQ by additive allelic rs3037354 effect in SAC male participants. For each unit increase in CTQ score, the CASI score is decreased by 0.20 (95% CI: 0.05–0.36, $p = 0.011$) points for each D allele (Figure 2).

Haplotype analyses

Our analyses revealed a high degree of linkage disequilibrium between the selected *NPY* SNPs in both Xhosa and SAC participants (Figure 3). We found no significant association between haplotype and CASI score for either population group. However, models examining the effect of the haplotype x CT interaction on CASI score revealed a significant interaction in Xhosa female participants ($p = 0.003$) (Table 5). Compared to the *C-I-A-A* reference haplotype, the rare haplotype representing eleven haplotypes with a combined frequency of 0.01, yielded a 0.53-point increase in CASI score per unit increase in CTQ ($p = 0.007$).

Table 4. *P*-values of SNP x CT Interaction Effects on CASI Scores Stratified According to Sex and Population.

		Xhosa				SAC			
		Genotype model		Allelic model		Genotype model		Allelic model	
Gene	SNP	Male	Female	Male	Female	Male	Female	Male	Female
NPY	rs16147	0.555	0.639	0.888	0.405	0.102	0.174	0.879	0.123
	rs3037354	0.367	0.156	0.924	0.834	0.069	0.534	0.011 *	0.364
	rs5573	0.577	0.556	0.688	0.326	0.183	0.115	0.727	0.183
	rs5574	0.539	0.806	0.688	0.819	0.670	0.203	0.095	0.043 *
ADCYAP1R1	rs2267735	0.820	0.946	0.140	0.880	0.518	0.305	0.231	0.219

SAC = South African Colored. The *p*-values for genotype x CTQ interaction tests under individual genotype and allelic models, stratified by group and sex, are shown. All models were adjusted for age, as well as STAI-T, CES-DC, CTQ, CD-RISC, A-COPE and AUDIT test scores. * indicates significant effects on CASI score (*p*-value < 0.05).

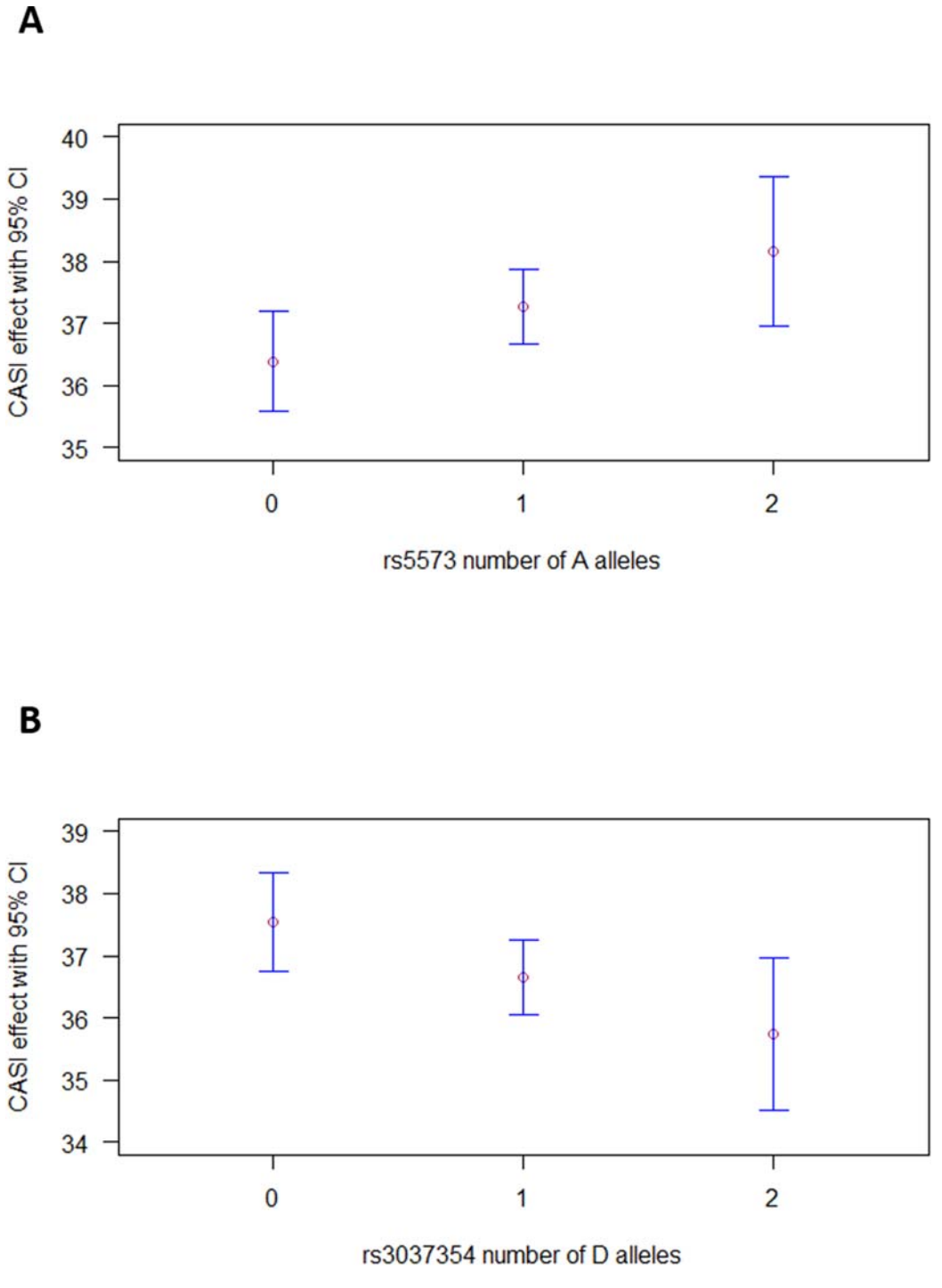

Figure 1. Allelic (number of minor alleles modeled) association plots showing estimated effects and their 95% confidence intervals on CASI scores. Plots show effects of the (a) rs5574 and (b) rs3037354 SNPs in Xhosa female participants.

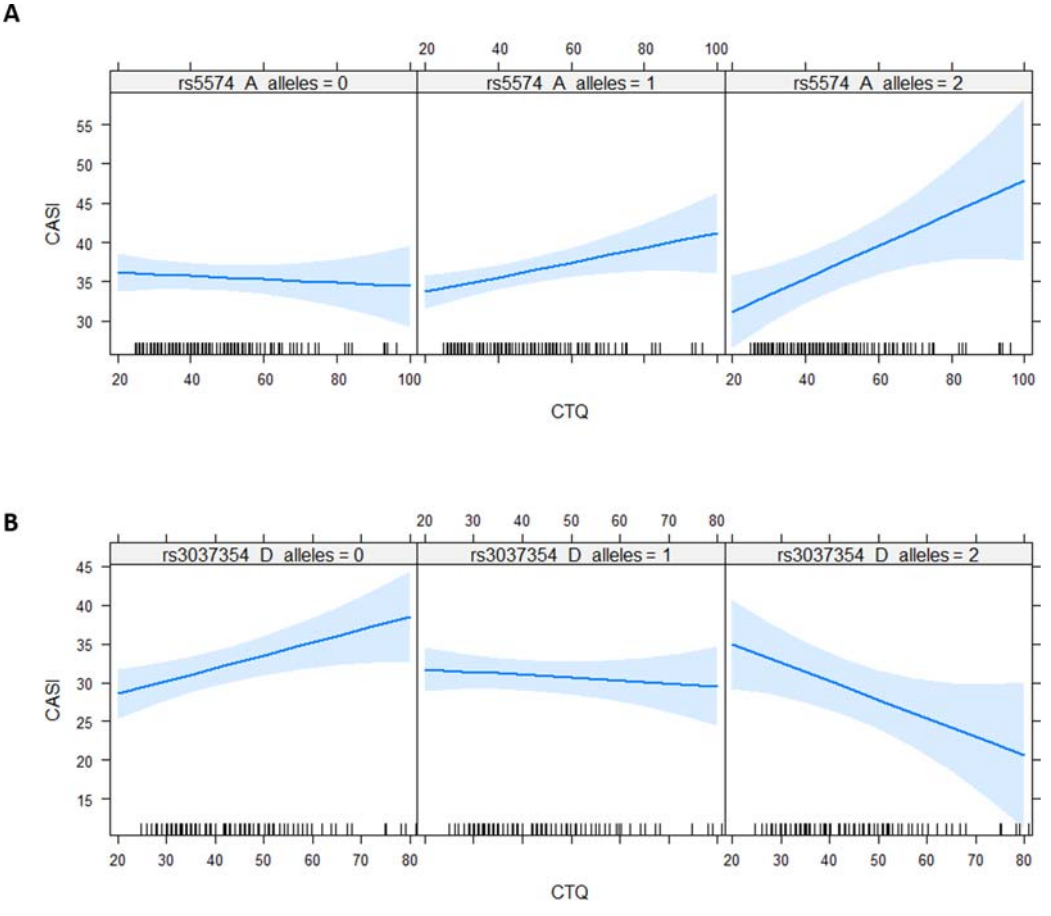

Figure 2. Plots illustrating significant SNP by CTQ score interaction effects on CASI scores. (a) The rs5574 by CTQ interaction effects on CASI scores in SAC female participants. Plots are stratified by the number of alleles harbored and show the estimated CASI versus CTQ for each possible number of alleles. The slopes of the lines show that for each unit increase in CTQ score, the CASI score is increased by 0.117 (95% CI: 0.05–0.36, $p = 0.011$) points for each A allele. (b) The rs3037453 by CTQ score interaction effects on CASI scores in SAC male participants. Plots are stratified by the number of deletion (D) variants harbored and show the estimated CASI versus CTQ for each possible number of D variants. The slopes of the lines show that for each unit increase in CTQ score, the CASI score is decreased by 0.20 (95% CI: 0.05–0.36, $p = 0.011$) points for each D allele. Plots include estimates and 95% confidence bands.

Table 5. Summary of NPY rs16147-rs3037354-rs5573-rs5574 Haplotype by CTQ Interaction Joint Effect on CASI Scores in Xhosa Female Participants.

rs16147	rs3037354	rs5573	rs5574	Haplotype frequency	Effect	Standard error	*P*-value
T	D	G	G	0.31	−0.04	0.03	0.250
T	I	G	G	0.28	0.04	0.04	0.252
C	I	A	G	0.04	0.10	0.06	0.107
C	I	G	G	0.02	0.09	0.13	0.493
^	^	^	^	0.01	0.53	0.19	0.007 *
C	I	A	A	0.33 #			

The effect size represents the difference between the CTQ x CASI slopes for the specific vs. reference haplotype. # reference haplotype. * highly significant effect ($p < 0.01$) on CASI score.

Discussion

We examined the role of gene-environment interactions in AS in a cohort of South African adolescents. As dysregulation of the stress response plays an important role in the pathophysiology of

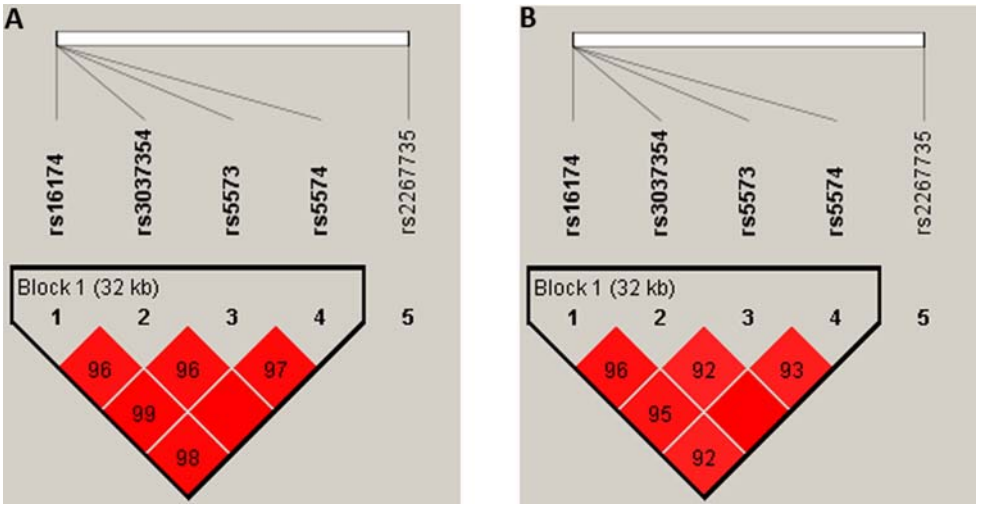

Figure 3. Linkage disequilibrium map of NPY for the (A) Xhosa and (B) SAC participants. D′ values are depicted in the diamonds.

anxiety-related disorders, we chose to investigate genetic variants in *NPY* and *ADCYAP1R1*, both of which play a role in HPA axis activity and thus influence the stress response. Though we hypothesized that genetic variation in the aforementioned genes would impact sensitivity to CT and thus mediate the development of AS, we did not have *a priori* hypotheses about the specific nature of these gene-environment interaction effects, and thus conducted a study that was explorative in nature.

Our analyses indicated that the D variant of rs3037354 is associated with reduced AS in Xhosa female participants. Using a cell culture model, Zhou et al. (2008) found this variant to be associated with increased NPY expression. Though their results failed to reach significance, increased NPY expression, and a corresponding increase in resilience to stress, would explain its apparently protective effect against AS. To the best of our knowledge, the rs5573 SNP has not been independently associated with anxiety. However, the A allele has been included in a seven-SNP haplotype associated with reduced NPY expression (Zhou et al., 2008), whilst the G allele has been included in a five-SNP haplotype associated with elevated plasma NPY (Zhang et al., 2012). This suggests that the A allele is associated with reduced NPY, supporting its association with increased AS in our Xhosa female participants.

In gene-environment interaction analyses, we found two significant *NPY* genotype x CT interactions. First, there was an intriguing interaction between rs3037354 and CT in SAC male participants, with increased experience of CT associated with decreased AS in individuals carrying the D variant. This counterintuitive result may be attributable to the fact that this polymorphism affects a glucocorticoid response element binding motif, with the D version associated with reduced glucocorticoid response and increased NPY transcription and secretion (Zhang et al., 2012). Accordingly, experience of CT in individuals carrying the D variant could increase NPY levels and in turn foster resilience in response to this stressor. The combination of CT and the rs5574 A allele was associated with increased CASI score in SAC female participants. Though we did not examine the effect of this SNP on NPY expression, the A allele has previously been included in a seven-SNP haplotype that was associated with lower NPY expression (Zhou et al., 2008) whilst the G allele was included in a five-SNP haplotype linked to increased NPY (Zhang et al., 2012). Reduced NPY has been linked with negative affect post stress exposure (Mickey et al., 2011) and increased amygdala activity in response to threatening faces (Domschke et al., 2010; Opmeer et al., 2014; Zhou et al., 2008), suggesting an inverse relationship between NPY levels and sensitivity towards the harmful effects of aversive stimuli.

Thus, if the rs5574 A allele is associated with reduced NPY expression, this may mediate the relationship we observed between CTQ and CASI scores.

Second, our significant CT x haplotype result in Xhosa females is more difficult to interpret, as the rare haplotype group essentially reflects the combination of eleven haplotypes rather than one specific genotype combination. However, if these rare haplotypes are predictive of reduced NPY expression, this would explain their acting as a risk factor for AS with increasing CT. Further research would be required to investigate this possibility.

Our results indicated no significant effects of the *ADCYAP1R1* SNP, rs2267735, on AS. Previous studies have indicated a sex-specific effect of this variant with C allele homozygosity in women associated with PTSD, heightened fear responsivity, increased bilateral amygdala and hippocampal activity in response to threatening faces, increased depressive symptoms, and reduced ADCYAP1R1 expression (Lowe et al., 2015; Ressler et al., 2011; Stevens et al., 2014). This difference is likely due to the rs2267735 SNP lying in a predicted estrogen response element i.e., an area of a gene where binding of activated estrogen receptors can change gene transcription (Ressler et al., 2011). Given the role of estrogen in *ADCYAP1R1* gene expression, the sex-dependent effects of this genotype on the stress response and behavior may only come into effect after puberty. Consequently, it is not possible to determine the influence of this estrogen-dependent effect in our study group, as we do not know whether reported CT occurred pre- or post-pubertally. Furthermore, as we asked participants to report on CT experienced before twelve years of age, it is probable that this developmental stage-dependency explains the lack of interaction between rs2267735 and CT on AS in our study group.

Certain limitations affect the interpretation of our results. First, though we recruited 951 adolescents, stratifying the analyses according to sex and ethnicity reduced the sample size per group and thus limited the power of the study to identify significant CT x genetic variant interactions, especially those of small effect. However, *a posteriori* analyses indicated that we achieved more than 80% power to detect a medium effect size ($f^2 = 0.15$) at an alpha value of 0.05. The achieved power varied within analysis subgroups and ranged from 89% in the ethnic group with the lowest sample size (SAC male) to 99% in the ethnic group with the largest sample size (Xhosa female). Based on this power calculation, we expect that these findings could be replicated in studies with a similar sample composition. Indeed, further research is needed to confirm the findings of this exploratory study. Second, we did not examine the effects of our *NPY* SNPs on gene expression and thus cannot stipulate whether such genetic variation influenced NPY levels. Third, though the four NPY SNPs included in our study are constituents of the seven-SNP functional haplotype reported by Zhou et al. (2008), it is not clear to what degree our haplotype captures the Zhou et al. haplotype. Further studies that examine linkage disequilibrium of NPY SNPs in populations of diverse ancestry would be required to assess this. Fourth, the relationship between stress, HPA axis activity, and NPY is complex with a bidirectional relationship between CRF and NPY. Therefore, both genetic variation in HPA axis genes and CT-induced changes in HPA axis physiology, which were not assessed in this study, could produce downstream effects on NPY signaling (Sajdyk et al., 2004). Furthermore, basic science studies suggest that the effects of stress on NPY release may alter with stressor duration (Thorsell et al., 1999) whilst the effect of *NPY* genotype on anxiety and HPA axis activity may depend on stressor severity (Amstadter et al., 2010; Witt et al., 2011). Thus, though the CTQ is a widely used, reliable and well-validated measure (Bernstein et al., 1994), it does not capture timing, duration, or cumulative exposure, parameters that are relevant in determining the effects of exposure. As the CTQ provides a retrospective measure of CT, it is possible that recall bias may affect these scores. Though different subtypes of childhood maltreatment are captured on the CTQ, we did not examine the effects of these subtypes in our analysis. We recommend that future studies in larger adequately powered samples undertake such stratified analyses. With respect to our study sample, our use of self-reported ethnicity is also potentially a limitation as it is unlikely to capture the genetic complexity inherent in each ethnic group. For this reason, future studies should examine ancestry informative markers in their analyses. AS as a

construct is inherently complex, contributed to by the interaction of multiple genetic variants in concert with environmental influences, and it is likely that as a phenotype it is not adequately captured by existing psychometric instruments (Zhou et al., 2008). Indeed, a recent twin study examining the genetic contributions to psychiatric traits in childhood and adolescence, found that a polygenic risk score explained a significant, though small, amount of the variance in anxiety measures at nine, fifteen and eighteen years of age (Taylor et al., 2018). Though such findings underscore the importance of gene-environment interactions in anxiety, it is likely that very large sample sizes will be required to consistently capture gene effects in complex behaviors such as AS (Zhou et al., 2008). Finally, though AS has been identified as a risk factor for the development of anxiety disorders, it is not possible to determine through such gene-environment interaction analysis what the clinical impact of increased or decreased CASI scores will be. However, previous research has found evidence for heightened risk even with modest increases in AS. For example, Hernandez Rodriguez et al. (2020) found that each unit increase in CASI score was associated with an 11.53-fold increase in the likelihood of meeting clinical levels of internalizing problems i.e., anxiety and depressive symptoms, six months later. We recommend that longitudinal studies that track AS and subsequent development of psychopathology in South African adolescents be conducted.

This study also has a number of strengths. First, we examined AS during adolescence, a sensitive developmental period during which the prevalence of anxiety disorders increases (Costello et al., 2011). Second, we included both male and female participants and performed parallel analyses for the two sexes, an important consideration given the estrogen-dependent effects of PACAP1R1 and evidence from basic science studies that NPY expression may differ between sexes (Jiménez-Vasquez et al., 2001; Ressler et al., 2011; Rugarn et al., 1999). Third, our participants belong to relatively understudied populations in psychiatric genetics. As considerable genetic diversity exists between populations, the results of genetic studies may not be broadly applicable across population groups. This is clearly born out in these results of this study, where sex and ethnicity effects were apparent. There is a clear need to undertake genetic studies in different populations if we are to better understand anxiety pathophysiology (Dalvie et al., 2015). This investigation contributes to a growing body of research examining the neurobiological underpinnings of risk and resilience in response to stress. This mechanistic knowledge is required to identify individuals likely to be in the greatest need of assistance and to target interventions appropriately (Enman et al., 2015). This remains an important consideration in countries such as South Africa where anxiety disorders often go undiagnosed and treatment resources are limited (Weinmann & Koesters, 2016).

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