Published in final edited form as:

Cogn Emot. 2017 August ; 31(5): 963–971. doi:10.1080/02699931.2016.1168779.

Partial replication of two rumination-related candidate gene studies

Carol A. Van Hulle^a, Sierra Clifford^b, Mollie N. Moore^a, Kathryn Lemery-Chalfant^a, and H. Hill Goldsmith^a

^aWaisman Center, University of Wisconsin–Madison, Madison, WI, USA

^bPsychology Department, Arizona State University, Tempe, AZ, USA

Abstract

Two recent papers associated candidate genes with brooding rumination, a possible cognitive endophenotype for depression, in children ages 8–14 years. Stone et al. reported that BDNF val66met polymorphism predicted brooding in adolescence. Woody et al. reported that children carrying at least one copy of a CRHR1 TAT haplotype reported less brooding than their peers in the presence of maternal depression. We attempted to replicate and extend these findings in a sample of twins aged 12–16 years. We analyzed the BDNF val66met (rs6265) polymorphism and two (rs242924 and rs7209436) out of three single nucleotide polymorphisms (SNPs) that Woody et al. used to create a CRHR1 haplotype. We controlled for maternal history of depression and clustering within families. Unlike Stone et al., we found higher brooding among BDNF Met carriers. This main effect was qualified by an interaction with pubertal status, with the effect driven by more physically mature participants. Similar to Woody et al., we found an interaction between CRHR1 SNPs and maternal depression, with the homozygous minor genotype acting as a protective factor against brooding in the presence of maternal depression. Findings provide partial support for the influence of candidate genes in two environmentally sensitive systems on brooding.

Keywords

Brooding; candidate gene; BDNF; CRHR1; depression; adolescence

Partial replication of two rumination-related candidate gene studies

There is a growing concern about the high number of false positives in published research, especially research using candidate gene methods (Duncan & Keller, 2011). Replication is key to strengthening confidence in the original positive results by generalising findings to related measures and/or different samples. Our aim was to replicate two recent studies linking rumination, a cognitive endophenotype for Major Depressive Disorder, to two putative candidate genes.

CONTACT Carol A. Van Hulle, cavanhulle@wisc.edu.

Disclosure statement

No potential conflict of interest was reported by the authors.

Major Depressive Disorder is one of the most common mental illnesses (World Health Organization, 2012). Although relatively rare in pre-adolescence (Fleming & Offord, 1990), by age 18 years, depression affects approximately 1 in 10 people (Avenevoli, Knight, Kessler, & Merikangas, 2008; Merikangas et al., 2010). Nolen-Hoeksema (1991) identified a cognitive response style (i.e. rumination or distraction) as a behavioural endophenotype for depression. In particular, higher levels of the maladaptive brooding style of rumination (Treyner, Gonzalez, & Nolen-Hoeksema, 2003) are associated with greater risk for onset of depression, longer duration, and increasing severity of symptoms (Abela & Hankin, 2011; Gibb, Grassia, Stone, Uhrlas, & McGeary, 2012). Brooding is the tendency to passively focus on one's self-distress in response to a stressful or upsetting situation. We have shown that brooding is heritable and shares most of its genetic influences with depression (Moore et al., 2013). Two recent studies based on a sample of $N = 228$ children ages 8–14 years old with depressed and non-depressed mothers reported associations between brooding and single nucleotide polymorphisms (SNPs) in two candidate genes for depression: *BDNF* (Stone, McGeary, Palmer, & Gibb, 2013) and *CRHR1* (Woody et al., 2015).

BDNF

The *BDNF* gene codes for the protein “brain-derived neurotrophic factor” which plays a role in the growth, differentiation, and maintenance of neurons (U.S. National Library of Medicine, 2015) and helps regulate synaptic plasticity (Peters, Dieppa-Perea, Melendez, & Quirk, 2010). One well-studied SNP in the *BDNF* gene is the valine to methionine substitution at codon 66 (val66met). Evidence indicates that *BDNF* val66met plays a role in depressive symptoms in children and adolescents (Dalton, Hammen, Najman, & Brennan, 2014) as well as cognitive response style (Hilt, Sander, Nolen-Hoeksema, & Simen, 2007; Stone et al., 2013). Of the relatively few studies examining the *BDNF* val66met polymorphism in relation to cognitive response style, two indicated that the Val/Val genotype was associated with higher rumination in late childhood and early adolescence (Hilt et al., 2007; Stone et al., 2013). First, Stone et al. found that the Val/Val genotype was associated with higher levels of brooding on the Child Response Style Scale (CRSS) among individuals aged 11–14 years ($N = 127$), controlling for current depression, although no significant effect of *BDNF* on rumination was observed among children aged 8–10 years of age ($N = 101$) or in the full sample. Second, Hilt et al. (2007) found that Val/Val homozygotes were at higher risk for brooding in a sample of girls aged 10–14 years ($N = 91$), but not in their mothers ($N = 95$, 52% with depression). In this study, *post hoc* analyses revealed a significant association between the Met allele and risk for rumination in the adult sample, but only among mothers with adult onset depression. Hilt et al. (2007) suggest that the risk allele for brooding may switch from the Val allele in adolescence to the Met allele in adulthood. This possibility is consistent with two studies that both found the Met allele to be associated with higher risk for brooding among adults with no history of depression, one small study of undergraduates ($N = 71$; Beevers, Wells, & McGeary, 2009) and one larger replication ($N = 273$; Clasen, Wells, Knopik, McGeary, & Beevers, 2011). Although these convergent results are promising, caution is still warranted in light of small samples, findings that only emerge in *post hoc* analysis, and differences across studies in how depression was accounted for and related to risk for rumination among Met carriers.

CRHR1

The *CRHR1* gene encodes the G-protein coupled type I corticotropin releasing hormone (CRH) receptor (CRHR1), which mediates the hormonal (i.e. HPA) and behavioural effects of CRH in response to many types of stress (Greetfeld et al., 2009; Steckler & Holsboer, 1999). A *TAT* haplotype comprising three SNPs in the *CRHR1* gene (rs7209436, rs110402, and rs242924) interacted with early stress (i.e. childhood maltreatment) in predicting levels of child and adult depressive symptoms (Bradley et al., 2008; DeYoung, Cicchetti, & Rogosch, 2011). One or two copies of the *TAT* haplotype acted as a protective factor against depression (although Laucht et al., 2013 reported *TAT* as a risk factor for depression in adults). Woody et al. (2015) found that possessing at least one copy of the *TAT* haplotype acted as a protective factor against brooding rumination, controlling for child depressive symptoms, but only among children whose mothers had a lifetime history of depression ($n = 72$).

Replication attempts

Candidate genotype–phenotype associations often fail to replicate. The National Cancer Institute working group on Replication in Association Studies (Chanock et al., 2007) offers several reasons. Small sample sizes in either the initial study or replication can lead to estimates of the effect size that differ substantially (whether significantly or not) from the true population effect size; that is, either the original or the replication effect size may be an outlier with small samples. Heterogeneity between samples, measures, or genetic variant under study can also reduce the chance of successful replication of positive findings. Replications can be either literal, operational, or constructive (Lykken, 1968). Literal and operational replications attempt to duplicate the original study in all its features and thus discover whether the original positive results might have been due to chance, error, or undetected bias. Constructive replication is a much stronger test of original positive results as it attempts to demonstrate generalisation of findings to related measures and/or different samples rather than attempting to duplicate the original design. Our aim was to constructively replicate the findings of Stone et al. (2013) and Woody et al. (2015) in a sample with approximately twice as many participants using the same SNPs (with one exception in a haplotype) and similar measures.

Methods

Sample

Participants were drawn from the Wisconsin Twin Project, a statewide, birth register-based twin sample that was mildly enriched for internalising and externalising symptoms when the twins were 7 years old (Schmidt et al., 2012). Families with twin births from 1989 to 2004 were invited to join the project. Eighty-one per cent of families contacted responded favourably. Families were invited to participate in an adolescent follow-up study. The University of Wisconsin Institutional Review Board approved this study. Parents provided consent, and adolescents assent, for their participation. All participants were paid. Our sample was composed of 770 adolescent twins aged 12–18 years ($M = 13.7$ $SD = 1.6$; 47% female). Primary caregiver reported participant race, ethnicity, family income, and parent

years of education. The sample was 93.1% Caucasian. Mothers' and fathers' average highest education level was 14.6 (SD = 2.3) and 14.0 (SD = 2.5) years, respectively, corresponding to some trade, tech, or college classes. The average combined family income category corresponds to \$60,000–\$70,000/year.

Seventy-one per cent ($n = 544$) of participants were genotyped for 78 SNPs spread over 15 genes as part of the larger Wisconsin Twin Project. We excluded 42 participants who had 10% or more missing information across all genotyped SNPs (calling the quality of their DNA into question). This criterion was selected because excluding individuals with 10% missing SNP data eliminated over 90% of the known genotyping errors in our sample (based on comparisons between genetically identical monozygotic (MZ) twins and within in a subsample of participants who were genotyped twice). We excluded 40 non-white, non-Hispanic participants. Eight participants were missing brooding rumination scores. Another 21 participants were missing maternal history of depression and 9 participants were missing additional covariates resulting in a reduced sample of $n = 441$ individuals. Gender was more evenly distributed in this reduced sample (50.3% female); average participant age was 13.3 years (SD = 1.4) and parent education was 14.8 (SD = 2.2) years for mothers and 14.1 (SD = 2.3) years for fathers. Average family income remained \$60,000–\$70,000/year.

Measures

Brooding—Adolescents completed the Ruminative Response Scale (RSS; Nolen-Hoeksema, 1991; Treynor et al., 2003) as part of an in-home interview. We averaged the five items comprising the brooding subscale to assess adolescents' tendency to engage in moody pondering (i.e. *What am I doing to deserve this?*) in response to being sad, down, anxious, or nervous. Adolescents rated the degree to which they generally engaged in each response on a 1 (*almost never*) to 4 (*almost always*) scale. Given the small number of items, the brooding subscale exhibited reasonable internal consistency ($\alpha = .71$).

DNA extraction and genotyping—DNA extraction from the buccal swabs was completed on MasterPure DNA 54 kits from Epicentre Biotechnologies at the Translational Genomics Research Institute (T-Gen) and at the University of Wisconsin–Madison Biotechnology Center (UWBiotech). Samples were stored at -80°C until DNA extraction. For SNP data, individuals were genotyped using either Sequenom (T-Gen) or KASPar (UWBiotech) sequencing technology, which employs mass spectrometry as well as second generation DNA sequencing, a technology that allows for the direct reading of the sequence of bases that make up the strands of DNA. Mass spectrometry sequencing is well equipped to identify mutations and SNPs (Edwards, Ruparel, & Ju, 2005). A total of 11 plates on Direct Lysis Plasmid96 DNA Purification Kits were used; each included two positive *Centre d'étude du polymorphisme humain* (CEPH) controls from the Utah reference sample (CEU) of the International HapMap Consortium (2005) and four randomly chosen intraplate replicates. CEPH controls were dispensed in two wells in each of the 11 plates to ensure a positive DNA control and to aid in future DNA target amplification. Each plex included a plate of CEPH trios to determine HAPMAP concordance and to identify Mendelian errors. Because our aim was to conduct replication analyses, we focused solely on the rumination-related SNPs identified by Stone et al. (2013) and Woody et al. (2015): the BDNF val66met

polymorphism (rs6265) and two of the three SNPs that form the *CRHR1* TAT haplotype (rs242924 and rs7209436). The third SNP in the haplotype was not genotyped in this sample; therefore, the two *CRHR1* SNPs were analysed separately. All three SNPs were confirmed to be in the Hardy–Weinberg equilibrium using the R package HardyWeinberg (Graffelman, 2008).

Adolescent depressive symptoms—Adolescents completed the Children’s Depression Inventory (CDI; Kovacs, 1985) orally during a telephone interview with a trained research assistant. The CDI is a 27-item questionnaire that measures negative mood, interpersonal difficulties, negative self-esteem, ineffectiveness, and anhedonia during the previous 2 weeks. The CDI has been shown to differentiate distressed from non-distressed children (Saylor, Finch, Spirito, & Bennett, 1984; Smucker, Craighead, Craighead, & Green, 1986). Adolescents rated each item on a 0–2 scale (response options depend on item content). Cohen’s alpha was .86. Since most adolescents endorsed few symptoms we log-transformed the CDI scores prior to analyses.

Pubertal status—Puberty was assessed using a composite score derived from mother- and self-reports on the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988) and self-report via the Picture-Based Interview About Puberty (PBIP; Dorn & Susman, 2002) collected during an in-home visit. The PDS consists of five questions about physical development (e.g. growth of body hair or breast development) scored from 1 (*has not begun*) to 4 (*is complete*). Trained interviewers administered the PBIP. Interviewers used a script and photographs to guide the adolescents through descriptions of the stages of pubertal development. The participant rated his or her stage of development in private. PDS ratings from both informants were converted to gonadal and adrenal status and composited with the PBIP using a method similar to that of Ellis, Shirtcliff, Boyce, Deardorff, and Essex (2011) resulting in a continuous puberty composite score that ranged from 1 (pre-pubertal) to 5 (post-pubertal). Participants were divided into those who have completed or nearly completed puberty (i.e. late puberty, pubertal status 4–5) and early- to mid-puberty (i.e. pubertal status 1–3; Rapkin, Tsao, Turk, Anderson, & Zeltzer, 2006).

History of maternal depression—Mothers completed the Composite International Diagnostic Index (CIDI 2.1; Robins et al., 1989) orally during a telephone interview with a trained research assistant. Lifetime depression diagnoses were collapsed across single episode depression (mild, moderate, or severe) and recurring depression (mild, moderate, or severe) into a presence/absence score.

Socio-economic status (SES) and age—We created an SES composite by averaging standardised maternal and paternal years of education and total family income. Age reflects adolescent’s age in months at the time of the telephone interview.

Data analysis plan

We used linear mixed models to account for nesting within families. Because our sample included MZ twins whose genes are identical, we also controlled for MZ status in all analyses. All analyses were conducted in IBM SPSSv22. Like Stone et al. (2013), we

compared *BDNF* Val/Val homozygotes (0; $n = 294$) to Met carriers (1; $n = 147$), and controlled for maternal history of depression in all analyses. *CRHR1* SNPs rs242924 and rs720936 were coded as major allele homozygotes (-1, $n = 157$ and 156), heterozygotes (0; $n = 194$ and 206), and minor allele homozygotes (1; $n = 90$ and 79). Because we did not genotype all three SNPs in the *CHRNA1* TAT haplotype, we analysed the two available *CRHR1* SNPs separately, first for main effects controlling for maternal depression, and then for the interaction with maternal depression. As recommended by Keller (2014), all analyses with genotype $\times$ trait interactions included maternal depression $\times$ genotype and maternal depression $\times$ trait interactions to properly control for the effect of maternal depression on the genotype $\times$ trait interaction.

Results

We did not find any sex differences outside the expected pubertal status difference (females reported more advanced pubertal status than males; $M = 3.3$ vs. 2.8 , $t = 6.5$, $p < .001$). Therefore, males and females were analysed together. Age and SES were not correlated with brooding ($r = .01$ and $-.05$, respectively) and were excluded from further analyses. Brooding ($M = 1.6$, $SD = 0.7$) and depression ($M = 6.4$, $SD = 6.0$) were moderately correlated ($r = .36$). Eighteen per cent of mothers ($n = 117$) reported a history of depression. Compared with offspring of non-depressed mothers, offspring of depressed mothers reported more brooding ($t = 3.5$, $p < .001$, Cohen's $d = .33$) and more depressive symptoms ($t = 2.3$, $p = .02$, Cohen's $d = .25$). There were no differences between MZ and dizygotic twins on any variables.

BDNF results

Participants who carried the Met version of *BDNF* val66met had higher brooding scores than those with the Val/Val genotype ($M = 2.1$, $SD = .60$ vs. $M = 1.9$, $SD = .60$, $p = .03$, effect size $r = .16$), controlling for maternal depression. Because our participants were older on average than the participants studied in Stone et al. (2013), and there is evidence that the risk allele is different for young adolescents and adults (Hilt et al., 2007), we conducted *post hoc* analyses to examine whether physical maturity could account for the findings. Slightly under half of our sample (43%) reported having completed or nearly completed puberty. The proportion of genotypes was approximately the same for both pubertal status groups (Fisher's exact test = .68). The interaction between *BDNF* allele and pubertal status was significant ($p = .03$), even though mature and less mature adolescents reported the same amount of brooding on average ($M = 2.0$ for both groups). As shown in Figure 1, Met carriers reported more brooding rumination among mature adolescents (effect size $r = .15$), whereas genotype had no effect on brooding among less mature adolescents (effect size $r = .00$). *Post hoc* tests (linear mixed models) of the main effect of genotype conducted separately for less mature and more mature individuals confirmed that possessing a copy of the Met allele was not related to brooding among less mature individuals ($p = .82$), but Met carriers reported significantly more brooding than non-met carriers among more mature individuals ($p = .008$). The main effect of *BDNF* on brooding and the interaction with pubertal status remained significant after controlling for adolescent depressive symptoms.

CRHR1 results

Like Woody et al. (2015), the main effect of CRHR1 SNP on adolescent brooding was non-significant (rs720936, $p = .22$; rs242924, $p = .32$), but both SNPs interacted with maternal depression in the direction reported by Woody and colleagues (Figure 2). The minor homozygote (*TT* for both SNPs) acted as a protective factor against brooding only among adolescents whose mothers reported a history of depression. The effect size for SNP rs720936 ($p = .01$) among those with a maternal history of depression was .33 vs. .08 among those without a maternal history of depression; for rs242924 ($p = .01$), the effect size was $r = .33$ vs. .001. These results were not driven by any asymmetry in the distribution of genotypes between children of depressed and non-depressed mothers. Unlike Woody et al., however, the interaction did not remain significant after controlling for adolescent depressive symptoms. A post hoc test showed a similar protective effect of *CRHR1* SNP against depressive symptoms among the group with a maternal history of depression that failed to reach significance after controlling for brooding. Thus, neither brooding nor depression alone drives the interaction. Over time, rumination contributes to the maintenance of depression (Nolen-Hoeksema, 1991), and most of the covariance between depression and brooding is due to shared genes. At these early adolescent ages, rumination and depression together may capture a level of genetic risk beyond either measure alone. In post hoc analyses, averaging standardised brooding and depressive symptoms resulted in a highly significant interaction between maternal history of depression and *CRHR1* SNPs ($p = .001$ for rs720936 and $p = .004$ for rs242924).

Discussion

We found a significant effect of *BDNF* val66met on rumination. However, we were unable to replicate the results reported by Stone et al. (2013). We found that the Met allele, rather than the Val/Val genotype, was associated with greater brooding. Our results are consistent with studies of non-depressed adults that find the Met allele to be associated with higher risk for rumination (Beevers et al., 2009; Clasen et al., 2011). Adult Met carriers may be vulnerable to rumination and depression via reductions in cortical surface (Legge et al., 2015) or white matter integrity (Carballedo et al., 2012, but see also Chiang et al., 2011). Indeed, post hoc analyses showed that the Met allele of the *BDNF* val66met SNP was a risk factor for the brooding style of rumination among more adult-like (i.e. more physically mature) adolescents. Thus, one possible explanation for this failure to replicate is the greater age range in our sample; nearly one-third of our participants were age 15 or older. At ages 8–14 years, few participants in Stone et al. were likely to have completed puberty.

This interpretation is challenged by the fact that Stone et al. (2013) found a stronger, rather than weaker, relation between Val/Val genotype and brooding among children in the upper age range in their sample. In addition, we were unable to find a significant relation between *BDNF* and rumination among less physically mature individuals who clustered around ages 11–12 years, whereas Stone et al. found the Val/Val genotype to be related to higher brooding among children in that same age range. Thus, although a maturation-related change in direction of effect is one explanation for discrepancies between studies, and one that deserves further consideration in developmental research, other explanations including

false positive findings must be taken into account. This is especially important given the post hoc nature of our *BDNF* by pubertal status interaction.

We were able to replicate the interaction between *CRHR1* and maternal depression reported by Woody et al. (2015). Replication of a specific interaction such as this is notable. We found that possessing a *TT* genotype in either *CRHR1* SNP rs242924 or rs720936 acted as a protective factor against depression among adolescents with a maternal history of depression. Woody et al. reported the same findings when testing each of the SNPs that form the *TAT* haplotype individually. However, unlike Woody et al., our results were not robust in the face of concurrent depressive symptoms. The relationship between depression and brooding is consistent across ages in our sample, making it unlikely the different results are due to participant age. Perhaps the inability to detect *CRHR1* after controlling for depression reflects the reduction in power from analysing each SNP separately rather than as part of a haplotype. In our sample, the strongest effects were found by combining depression and brooding remains a comorbid condition and may intensify the effect of environmental influences (i.e. maternal depression) in the face of genetic vulnerability.

Numerous similarities exist between our sample and those analysed by Stone et al. and Woody et al. Both our participants and those analysed in Stone et al. and Woody et al. were similar in terms of race/ethnicity, maternal education, income level, and average depressive symptoms among the adolescents. Our frequency of Val/Val vs. Met carriers is similar to that reported by Stone et al., although Woody et al. reported only *TAT* vs. no *TAT* frequencies, preventing direct comparison. Slightly fewer participants in our sample definitely lacked a *TAT* haplotype (i.e. homozygous AA for both SNPs), 35% vs. 45% reported by Woody et al. The measures used to assess brooding by us (RSS) and by Stone et al. and Woody et al. (CRSS) were developed to tap the same cognitive response styles identified by Nolen-Hoeksema (Treynor et al., 2003; Ziegert & Kistner, 2002). Thus, our study approximates the other studies in terms of sample, measures, and genetics, and thus can be viewed as a valid constructive replication.

Although we were able to confirm the roles of the *BDNF* and *CRHR1* candidate genes in brooding rumination with our independent constructive replication, this study has some of the same limitations that characterise the candidate gene literature as a whole. The first is the expected small effect size of any one candidate gene. With small effect sizes, the risk of type 1 errors is elevated, especially when conducting a large number of tests. With a typical genome wide association study, for example, over one million logistic regressions are performed, with greater than 50,000 type 1 errors estimated without correction of p values. In our case, we only tested three SNPs that represent two candidate genes to minimise the type 1 error risk. On the flip side is the risk of type II errors, especially with smaller sample sizes. Although our replication sample was larger than the initial studies, a much larger sample size in the thousands would increase confidence in the findings. A second concern is that candidate gene studies do not control for linkage disequilibrium with other SNPs unless specifically chosen for that purpose (i.e. designing SNP sets to capture maximum genetic variability). In other words, the val66met may not impact brooding directly but may be in tight linkage disequilibrium with a true risk allele. A third concern is that covariates could be influencing the reported associations. In our case, we examined age, pubertal status, sex, and

family SES as possible covariates – testing main effects and bivariate interactions with the main predictors (Keller, 2014), with puberty emerging as a potentially important factor. Unmeasured genetic and environmental third variables could also account for the reported associations. Although we focused on two candidate genes because our study was a replication, there are likely other genes that also influence risk. Fourth, we coded the BDNF genotypes dominantly in line with Mendel's third law of genetics (i.e. dominance; recessive alleles will be masked by dominant alleles), the previous literature, and the studies we were attempting to replicate. Fifth, although encouraging, our planned results do not survive correction for multiple testing, with the exception of our *post hoc* analyses with the brooding-depression composite and effect of Met carriers among more mature individuals which do fall below the false discovery rate (Glickman, Rao, & Schultz, 2014) when all planned and *post hoc* analyses are included. Finally, the interaction between CRHR1 and Maternal Depression was driven by the smallest cell (individuals homozygous for minor allele and with depressed mothers), and as such, should be interpreted cautiously. This is a common pattern in candidate gene $\times$ environment research, and it is problematic because results may be disproportionately influenced by a small number of extreme cases which may reflect chance alone. At the same time, it is reasonable to expect that the individuals at the most risk, genetically and environmentally, would be the ones to drive an interaction. Replication in a larger sample with a sufficient number of cases in even the smallest cells is needed before strong conclusions can be drawn.

Since we did not find any relationship between brooding rumination and age, SES, or sex, we did not include them as covariates. The age-related trajectory of brooding has not yet been characterised nor do studies typically include SES as a covariate. Evidence for sex differences in rumination is mixed. A recent meta-analysis suggests that sex differences in rumination emerge in early- to mid-adolescence (Rood, Roelofs, Bögels, Nolen-Hoeksema, & Schouten, 2009), possibly due to increases in feminine role identification (Broderick, 2004; Cox, Mezulis, & Hyde, 2010). Meta-analyses of adolescent (Rood et al., 2009) and adult samples (Johnson & Whisman, 2013), indicate that gender differences, though significant, are small in magnitude.

Our results generally support the role of CRHR1 in the development of depression via the brooding style of rumination. Findings for BDNF are more equivocal. We were unable to replicate the relation between the Val/Val genotype and rumination found by Stone et al. (2013) in an early adolescent sample, but our findings in an early to mid-adolescent sample are consistent with past research suggesting that the direction of effect depends on maturation. However, like Stone et al. (2013) and Woody et al. (2015), we did not assess brooding and depression longitudinally, a necessary step in determining if genetic associations with brooding precede their associations with depression. A longitudinal study from childhood through late adolescence would also allow a stronger test of the hypothesis that the BDNF risk allele for rumination and depression switches from Val to Met during adolescence (e.g. Hilt et al., 2007).

Acknowledgments

We thank the families who participated in this study and the staff members who helped with the recruitment and data collection.

Funding

This work was supported by NIMH [R01 MH59785 and P50 MH069315]. Infrastructure was supported by the Waisman center via NICHD [P30 HD03352].

References

- Abela JRZ, Hankin BL. Rumination as a vulnerability factor to depression during the transition from early to middle adolescence: A multiwave longitudinal study. *Journal of Abnormal Psychology*. 2011; 120(2):259–271. [PubMed: 21553940]
- Avenevoli, S., Knight, E., Kessler, RC., Merikangas, KR. Handbook of depression in children and adolescents. In: Abela, JRZ., Hankin, BL., editors. *Epidemiology of depression in children and adolescents*. New York, NY: Guilford Press; 2008. p. 6-32.
- Beevers CG, Wells TT, McGeary JE. The BDNF val66met polymorphism is associated with rumination in healthy adults. *Emotion*. 2009; 9(4):579–584. [PubMed: 19653783]
- Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, Ressler KJ. Influence of child abuse on adult depression: Moderation by the corticotropin-releasing hormone receptor gene. *Archives of General Psychiatry*. 2008; 65(2):190. [PubMed: 18250257]
- Broderick PC. A prospective study of rumination and depression in early adolescence. *Clinical Child Psychology and Psychiatry*. 2004; 9(3):383–394.
- Carballedo A, Amico F, Ugwu I, Fagan AJ, Fahey C, Morris D, Frodl T. Reduced fractional anisotropy in the uncinate fasciculus in patients with major depression carrying the met-allele of the val66met brain-derived neurotrophic factor genotype. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2012; 159B(5):537–548.
- Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Collins FS. Replicating genotype-phenotype associations. *Nature*. 2007; 447(7145):655–660. [PubMed: 17554299]
- Chiang MC, Barysheva M, Toga AW, Medland SE, Hansell NK, James MR, Thompson PM. BDNF gene effects on brain circuitry replicated in 455 twins. *Neuroimage*. 2011; 55(2):448–454. [PubMed: 21195196]
- Clasen PC, Wells TT, Knopik VS, McGeary JE, Beevers CG. 5-HTTLPR and BDNF val66met polymorphisms moderate effects of stress on rumination. *Genes, Brain and Behavior*. 2011; 10(7):740–746.
- Cox SJ, Mezulis AH, Hyde JS. The influence of child gender role and maternal feedback to child stress on the emergence of the gender difference in depressive rumination in adolescence. *Developmental Psychology*. 2010; 46(4):842–852. [PubMed: 20604606]
- Dalton ED, Hammen CL, Najman JM, Brennan PA. Genetic susceptibility to family environment: BDNF val66met and 5-HTTLPR influence depressive symptoms. *Journal of Family Psychology*. 2014; 28(6):947–956. [PubMed: 25347540]
- DeYoung CG, Cicchetti D, Rogosch FA. Moderation of the association between childhood maltreatment and neuroticism by the corticotropin-releasing hormone receptor 1 gene: CRHR1 moderation of neuroticism in maltreatment. *Journal of Child Psychology and Psychiatry*. 2011; 52(8):898–906. [PubMed: 21438878]
- Dorn, L., Susman, E. Puberty script: Assessment of physical development in boys and girls. Cincinnati, OH: Cincinnati Children's Hospital Medical Center; 2002.
- Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry*. 2011; 168(10):1041–1049. [PubMed: 21890791]
- Edwards JR, Ruparel H, Ju J. Mass-spectrometry DNA sequencing. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2005; 573(1–2):3–12. [PubMed: 15829234]

- Ellis BJ, Shirtcliff EA, Boyce WT, Deardorff J, Essex MJ. Quality of early family relationships and the timing and tempo of puberty: Effects depend on biological sensitivity to context. *Development and Psychopathology*. 2011; 23(1):85–99. [PubMed: 21262041]
- Fleming JE, Offord DR. Epidemiology of childhood depressive disorders: A critical review. *Journal of the American Academy of Child & Adolescent Psychiatry*. 1990; 29(4):571–580. [PubMed: 2201675]
- Gibb BE, Grassia M, Stone LB, Uhrlass DJ, McGeary JE. Brooding rumination and risk for depressive disorders in children of depressed mothers. *Journal of Abnormal Child Psychology*. 2012; 40(2): 317–326. [PubMed: 21826445]
- Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. *Journal of Clinical Epidemiology*. 2014; 67(8):850–857. [PubMed: 24831050]
- Graffelman, J. HardyWeinberg (Version 1.3) [R]. 2008. Retrieved from <http://127.0.0.1:21293/library/HardyWeinberg/html/HWChisq.html>
- Greetfeld M, Schmidt MV, Ganea K, Sterlemann V, Liebl C, Muller MB. A single episode of restraint stress regulates central corticotrophin-releasing hormone receptor expression and binding in specific areas of the mouse brain. *Journal of Neuroendocrinology*. 2009; 21(5):473–480. [PubMed: 19302188]
- Hilt LM, Sander LC, Nolen-Hoeksema S, Simen AA. The BDNF val66met polymorphism predicts rumination and depression differently in young adolescent girls and their mothers. *Neuroscience Letters*. 2007; 429(1):12–16. [PubMed: 17959306]
- Johnson DP, Whisman MA. Gender differences in rumination: A meta-analysis. *Personality and Individual Differences*. 2013; 55(4):367–374. [PubMed: 24089583]
- Keller MC. Gene $\times$ environment interaction studies have not properly controlled for potential confounders: The problem and the (simple) solution. *Biological Psychiatry*. 2014; 75(1):18–24. [PubMed: 24135711]
- Kovacs M. The Children's Depression, Inventory (CDI). *Psychopharmacology Bulletin*. 1985; 21(4): 995–999. [PubMed: 4089116]
- Laucht M, Treutlein J, Blomeyer D, Buchmann AF, Schmidt MH, Esser G, Banaschewski T. Interactive effects of corticotropin-releasing hormone receptor 1 gene and childhood adversity on depressive symptoms in young adults: Findings from a longitudinal study. *European Neuropsychopharmacology*. 2013; 23(5):358–367. [PubMed: 22748421]
- Legge RM, Sendi S, Cole JH, Cohen-Woods S, Costafreda SG, Simmons A, Fu CHY. Modulatory effects of brain-derived neurotrophic factor val66met polymorphism on prefrontal regions in major depressive disorder. *The British Journal of Psychiatry*. 2015; 206(5):379–384. [PubMed: 25745134]
- Lykken DT. Statistical significance in psychological research. *Psychological Bulletin*. 1968; 70(3p1): 151–159. [PubMed: 5681305]
- Merikangas KR, He J, Burstein M, Swanson SA, Avenevoli S, Cui L, Swendsen J. Lifetime prevalence of mental disorders in U.S. adolescents: Results from the National Comorbidity Survey Replication-Adolescent supplement (NCS-A). *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010; 49(10):980–989. [PubMed: 20855043]
- Moore MN, Salk RH, Van Hulle CA, Abramson LY, Hyde JS, Lemery-Chalfant K, Goldsmith HH. Genetic and environmental influences on rumination, distraction, and depressed mood in adolescence. *Clinical Psychological Science*. 2013; 1(3):316–322. [PubMed: 23956956]
- Nolen-Hoeksema S. Responses to depression and their effects on the duration of depressive episodes. *Journal of Abnormal Psychology*. 1991; 100(4):569–582. [PubMed: 1757671]
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of fear extinction with hippocampal-infra-limbic BDNF. *Science*. 2010; 328(5983):1288–1290. [PubMed: 20522777]
- Petersen AC, Crockett L, Richards M, Boxer A. A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence*. 1988; 17(2):117–133. [PubMed: 24277579]

- Rapkin AJ, Tsao JCI, Turk N, Anderson M, Zeltzer LK. Relationships among self-rated tanner staging, hormones, and psychosocial factors in healthy female adolescents. *Journal of Pediatric and Adolescent Gynecology*. 2006; 19(3):181–187. [PubMed: 16731411]
- Robins LN, Wing J, Wittchen H-U, Helzer JE, Babor TF, Burke J, Towle LH. The composite international diagnostic interview: An epidemiologic instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Archive of General Psychiatry*. 1989; 45(12):1069–1077.
- Rood L, Roelofs J, Bögels SM, Nolen-Hoeksema S, Schouten E. The influence of emotion-focused rumination and distraction on depressive symptoms in non-clinical youth: A meta-analytic review. *Clinical Psychology Review*. 2009; 29(7):607–616. [PubMed: 19682781]
- Saylor CF, Finch AJ, Spirito A, Bennett B. The children's depression inventory: A systematic evaluation of psychometric properties. *Journal of Consulting and Clinical Psychology*. 1984; 52(6):955–967. [PubMed: 6520288]
- Schmidt NL, Van Hulle CA, Brooker RJ, Meyer LR, Lemery-Chalfant K, Goldsmith HH. Wisconsin twin research: Early development, childhood psychopathology, autism, and sensory over-responsivity. *Twin Research and Human Genetics*. 2012; 16(01):376–384. [PubMed: 23200241]
- Smucker MR, Craighead WE, Craighead LW, Green BJ. Normative and reliability data for the children's depression inventory. *Journal of Abnormal Child Psychology*. 1986; 14(1):25–39. [PubMed: 3950219]
- Steckler T, Holsboer F. Corticotropin-releasing hormone and receptor subtypes of emotion. *Biological Psychiatry*. 1999; 46:1480–1508. [PubMed: 10599478]
- Stone LB, McGeary JE, Palmer RHC, Gibb BE. Identifying genetic predictors of depression risk: 5-HTTLPR and BDNF val66met polymorphisms are associated with rumination and co-rumination in adolescents. *Frontiers in Genetics*. 2013; 4
- The International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005; 437(7063):1299–1320. [PubMed: 16255080]
- Treynor W, Gonzalez R, Nolen-Hoeksema S. Rumination reconsidered: A psychometric analysis. *Cognitive Therapy and Research*. 2003; 27(3):247–259.
- U.S. National Library of Medicine. Genetics home reference. Jul 13. 2015 Retrieved from <http://ghr.nlm.nih.gov/gene/BDNF>
- Woody ML, Kudinova AY, McGeary JE, Knopik VS, Palmer RHC, Gibb BE. Influence of maternal depression on children's brooding rumination: Moderation by CRHR1 TAT haplotype. *Cognition and Emotion*. 2015:1–13.
- World Health Organization. Depression fact sheet. Oct. 2012 Retrieved from <http://www.who.int/mediacentre/factsheets/fs369/en/>
- Ziegert DI, Kistner JA. Response styles theory: Downward extension to children. *Journal of Clinical Child & Adolescent Psychology*. 2002; 31(3):325–334. [PubMed: 12149970]

Figure 1.
Mean brooding rumination by *BDNF* val66met genotype and pubertal status.

Figure 2.

Mean brooding rumination by CRHR1 genotype and maternal lifetime history of depression.

Notes: Frequencies for participants with no maternal depression: rs720936 TT = 54, CT = 152, CC = 102 and rs242924 TT = 60, GT = 147, GG = 101; frequencies for participants with maternal depression: rs720936 TT = 25, rs720936 CT = 54, CC = 54, and rs242924 TT = 30, GT = 57, GG = 56.