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Age and Puberty Moderation of Genetic and Environmental Influences On Self- and Parent-Reported Internalizing Symptoms

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Age and Puberty Moderation of Genetic and Environmental Influences On Self- and Parent-Reported Internalizing Symptoms

by

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Dedication

This work is dedicated to my cherished pup, Dog—my constant companion and source of support for over 15 years. You keep me grounded, loved, and listened to, regardless of your deaf ears.

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Abstract

Age and Puberty Moderation of Genetic and Environmental Influences On Self- and Parent-Reported Internalizing Symptoms

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Anxiety symptoms and depression symptoms commonly co-occur and may both be influenced by the same genetic risks. Previous research also suggests that genetic influences on internalizing symptoms might shift as children age into adolescence. Additionally, the initiation of gender differences in the prevalence of anxiety and depression during adolescence has implicated puberty as playing a role in the development of internalizing disorders. The current study examined genetic and environmental influences unique to and shared between anxiety and depression as moderated by age and by puberty to determine how the etiology of internalizing disorders, and their co-occurrence, changes with development.

We analyzed the data from a sample of 1,031 twins from the Texas Twin Project that range in age from 8 to 20 years ($M = 13.4$, $SD = 2.9$). Using twin-report, parent-report, and a combined-reporter composite on internalizing symptoms, three separate common and specific pathways biometric twin models for anxiety and depression were fit. These were then moderated by age, by pubertal status, and by both age and pubertal status

simultaneously to determine if potential developmental shifts in genetic and environmental influences on anxiety and depression varied as a function of age, pubertal status, or both.

Significant developmental trends in the etiology specific to anxiety, to depression, and broad across internalizing were not consistent across reporters. Specific to anxiety, there were genetic increases with age in the combined report models. Specific to depression, there were genetic increases by parent-report with both age and pubertal status. For broad internalizing, there were genetic increases with age by parent-report, and shared environmental decreases with age by twin report. No significant trends were detected in any model when both age and puberty were simultaneously entered as moderators, suggesting that these developmental trends are not of sufficient magnitude to distinguish between the effects of chronological age or pubertal status.

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Internalizing Problems in Puberty

Internalizing problems are one broad class of mental health issues faced by adolescents, characterized by emotion dysregulation and pronounced negative affectivity, resulting in symptoms such as depressed mood, fear, and worry (Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). The emergence of these symptoms during childhood and adolescence precedes the occurrence of anxiety and depressive disorders throughout the lifespan (Pine, Cohen, Gurley, Brook, & Ma, 1998). These developmental trends indicate a need to understand the etiology of internalizing problems in early life. The median age of onset for anxiety occurs earlier in life, approximately 6 years of age, and by early adolescence lifetime prevalence of any anxiety disorder is about 30%, similar to adult prevalence rates (Kessler et al., 2005; Merikangas et al., 2010). For depressive disorders, prevalence increases sharply in adolescence (15-17%) from relatively rareity in childhood (2-8%), and by late adolescence prevalence is comparable to adult rates of depression (Merikangas, Nakamura, & Kessler, 2009; Merikangas et al., 2010; Zahn-Waxler et al., 2000). The timing of these developments in early adolescence highlights the potential role of puberty as a period in which the genetic and environmental etiology of internalizing problems may shift, as environmental influences on children change, or along with potential puberty related genetic activation. The current study investigates potential changes in genetic and environmental influences on anxiety and depression as a function of age and as a function of puberty to determine first, whether there are developmentally related trends in the etiology of anxiety and depression, and second, the extent that potential developmental changes in internalizing are driven by puberty above and beyond chronological age.

PUBERTY AS A DEVELOPMENTALLY SENSITIVE PERIOD

Puberty encompasses a diverse array of phenomena centered around the activation of gonadal hormones resulting in sexual maturity, and includes not only physical changes, but also emotional, cognitive and resulting psychosocial shifts. These changes occur in all individuals, though individual differences are present in hormone levels, pubertal timing, and pubertal tempo. As such, though measures of these pubertal indicators may be considered risk factors, puberty itself may instead be considered a developmentally sensitive period. Increases in internalizing psychopathology during adolescence indicate that the myriad developmental and psychosocial changes associated with this transition may exert an influence on internalizing. These changes occur both internally and externally, for example activation of pubertal hormones, and increasingly demanding and complex responsibilities and social roles.

Hormonal changes during puberty play a significant role in adolescent brain development, as steroid hormones associated with puberty carry out effects on neural circuitry changes during this period (Sisk & Zehr, 2005). Hormones associated with puberty have also been associated with internalizing symptoms, and this occurs across the lifespan and across species (Schulz, Molenda-Figueira, & Sisk, 2009; Young, Midgley, Carlson, & Brown, 2000). In male rodents, testosterone plays an organizational role for anxiogenic behaviors, and in females, estrogen receptors have been linked to anxiety (Imwalle, Gustafsson, & Rissman, 2005; Sisk & Zehr, 2009). Life periods marked by hormonal variability in women, including menarche in early puberty, are marked with increases in internalizing problems (Halbreich & Kahn, 2001). Changes in estrogen have been linked with depression and anxiety, and may underlie women's greater vulnerability to internalizing type disorders over men (Young & Korszun, 2002). Furthermore, the association between puberty status and depression in girls has been better accounted for by

levels of hormones such as estradiol and testosterone than by morphological changes that occur with puberty, as measured by Tanner stage (Angold, Costello, Erkanli, & Worthman, 1999).

Changes in the environment also occur as children transition from childhood into adolescence across a wide variety of settings, including school and home settings, and peer and adult interactions. Though research reveals mixed findings on the association between puberty and internalizing in boys (Reardon, Leen-Feldner, & Hayward, 2009), particularly early maturing girls appear to be more vulnerable to social stressors, and at least partially accounts for associations between pubertal timing and internalizing symptoms (Reynolds & Juvonen, 2010). In a study measuring cortisol reactivity to an interpersonal stress task, pubertal timing was associated with internalizing symptoms in both boys and girls, however stress reactivity mediated with relationship only for girls (Natsuaki et al., 2009). Taken together, the literature supports a complex and dynamic relationship between outside the skin influences—environmental—and inside the skin changes—hormonal variation—that occur with puberty and risk for internalizing problems.

Furthermore, the emergence of sex-differences in the prevalence of internalizing disorders occurs in adolescence, particularly with depression, with women twice as likely to present with depression than men after age 15 (Moffit et al., 2007). As such, puberty has been implicated as playing a role in internalizing presentations given the differential experiences and changes affecting boys and girls during this developmental period (Angold, Costello, & Worthman, 1998; Brooks-Gunn, 1984). In depression, pubertal status as measured by Tanner Stage predicted the occurrence of the sex differential in prevalence above and beyond age or pubertal timing, with mid-puberty marking the period when girls begin to surpass boys in reports of depression (Angold et al., 1998). Additionally, pubertal status is generally related to increased anxiety symptoms in girls, though less so for boys

(Reardon, et al., 2009). During this time, youth may be more vulnerable to potentially damaging experiences, both indogenously and exogenously, and these affects may be more pronounced for girls.

BEHAVIOR GENETICS AND INTERNALIZING

Behavior genetic studies allow for examination of both genetic and environmental influences, shedding light on these complex and dynamic influences. By capitalizing on known degrees of genetic difference within family members, behavior genetic studies parse out the variability in a trait into additive genetic (A), shared environmental (C), and non-shared environmental influence (E). In adulthood, estimates of heritability of generalized anxiety and major depression from meta-analyses are approximately 32% and 37% respectively (Hettema, Neale, & Kendler, 2001; Sullivan, Neale, & Kendler, 2000). In childhood and adolescence, estimates have been variable depending on reporter, age, and measured construct (Eley, 1999). Some studies show minimal heritability on anixety and greater shared environmental influence earlier in life (Eley & Stevenson, 1999), which is relatively minimal in adulthood, though other studies demonstrate higher heritability estimates in childhood, approximately 59% for anxiety and 75% for depression (Tharpar & McGuffin, 1998). This indicates that reporter difference are an important consideration in child and adolescent studies of internalizing, but may also indicate developmental changes in etiology.

Anxiety and depression are both conceptually linked by negative affectivity or internalizing syndrome, and show both high sequential and contemporaneous comorbidity during the lifetime (Clark & Watson, 1991; Moffit et al., 2007). Additionally, this comorbidity is largely genetic, with the genetic correlation as high as 1.0 in adult women (.74 in men; Kendler, Gardner, Gatz, & Pedersen, 2007). In youth, shared heritability

accounts for over 50-80% of the variance in anxiety and depression (Eley & Stevenson, 1998; Tharpar & McGuffin, 1997). The difference between these estimates of genetic influence on comorbidity may suggest that genetic influences common to internalizing disorders may be more pronounced in adulthood than earlier in life.

This genetic epidemiological evidence indicates the potential for fluctuations in genetic and environmental etiology shared between and specific to anxiety and depression throughout development. Of studies that have evaluated developmental changes in anxiety and depression, one meta-analysis demonstrated linear increases in heritability of anxiety and depression from childhood to adulthood, though at different rates (Bergen, Gardner, & Kendler, 2007). One longitudinal twin study, focused specifically in adolescence, indicated that earlier measures of pubertal maturation moderated environmental influences on later depressive symptoms at age 14 in girls, and age 17 in boys (Edwards, Rose, Kaprio, & Dick, 2011). In addition, an examination of genetic influences on anxiety and depression across adolescence indicated that genetic factors important during late childhood attenuate during adolescence as other sets of genetic influence became predominant (Kendler, Gardner, & Lichentenstein, 2008). Genetic activation of risk during puberty, as noted by increasing heritability during this time, has been found for rule-breaking externalizing symptoms, as well as in eating disorder behavior (Harden et al., 2015; Klump et al., 2007), leaving open the possibility for a similar phenomenon to be present with internalizing.

The goals of the current study are to investigate whether the genetic and environmental etiology of internalizing symptoms may differ between childhood and adolescence, both specific to and shared between anxiety and depression. Furthermore, we evaluate if these potential developmental trends in etiology occur as a function of chronological age or pubertal maturation. Given previous epidemiological data suggesting puberty as a time of increased internalizing problems, and behavior genetic work indicating

genetic factors influencing psychopathology come on-line during puberty, we predicted a genetic activation in risk associated with puberty, as indicated by increasing heritability as a function of pubertal status above and beyond age.

Methods

PARTICIPANTS

Our current sample of twins comes from the Texas Twin Project, a study of school-aged twins in the central Texas area (Harden, Tucker-Drob, & Tackett, 2013). Twins in grades 3 though 12 were recruited using directory information obtained from public schools and invited to participate in an in-lab study, which included an extensive computerized survey, cognitive assessments, behavioral tasks, and saliva and hair samples to assess pubertal and stress hormones. In addition to the twins' participation, parents were also invited to complete an online survey. The subsample used in these analyses includes 1,031 individuals from 547 pairs (178 monozygotic [MZ], 369 dizygotic [DZ]; 50.2% female) ages 8 to 20 years (M = 13.4, SD = 2.9), and parent report from 454 families. This sample includes 20 triplet sets contributing three pairs each, and one family with two sets of twins. Our sample is racially and socioeconomically diverse, and representative of the surround area, with approximately one-third of the families reporting having ever received public assistance. Sixty-eight percent of the same is white, 20% Hispanic/Latino, 10% African-American, and 2% other race or ethnicity.

MEASURES

Zygosity

Zygosity for same sex twin pairs was classified using a latent-class analysis (LCA) of survey items regarding twins' similarity. Opposite sex twin pairs were all classified as DZ. Parents and two trained research assistants rated twins on five items of physical similarity (e.g. "facial appearance"), as well as difficulty in telling them apart. For twin pairs over age 14, each twin reported on these items as well. Zygosity classification by

LCA has previous been shown to have high concordance with zygosity as determined by molecular genetic assessment (Rietveld et al., 2000).

Internalizing

Measures of anxiety and depression were obtained via youth self-report and parent report on an abbreviated version of the Achenbach Child Behavior Checklist (CBCL; Achenbach, 1991; Lizotte, Chard-Wierschem, Loeber, & Stern, 1992). Parents and twins rated their agreement with each item on a 3-point scale from 0 – *Not true* to 2 – *Very true or often true*. A series of exploratory and confirmatory factor analyses were fit separately to twin and parent report items to determine the items to be included in an anxiety and a depression composite score from the Anxious/Depressed and Withdrawn/Depressed internalizing CBCL subscales. Five items from the twin-report were included for the anxiety composite (M = .64, SD = .48, α = .76), including, "I am nervous or tense," and "I worry a lot." Six items from the twin-report were included for the depression composite (M = .35, SD = .36, α = .69), including, "There is very little that I enjoy," and "I am unhappy, sad, or depressed." Parent report included four items for anxiety (M = .36, SD = .38, α = .69), including, "too fearful or anxious" and "self-conscious or easily embarrassed." Five items were included from parent report for the depression composite (M = .16, SD = .26, α = .66), including "cries a lot," and "withdrawn, doesn't get involved with others."

Puberty

Pubertal status was measured using the Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988), in which twins rate themselves on a variety of physical changes that accompany puberty. Two items were only administered to males, assessing facial hair growth and deepening of voice; and two items were only administered

to females rating breast growth and whether menarche was reached. Both sexes responded to three items on development in height, body changes, and skin. Twin rated their development on a 4-point scale from 1 – *Not yet begun to change* to 4 – *Finished changing* (reached menarche was recoded as 1 – *Not reached*, 4 – *Reached*). Mean scores of the sex appropriate items were devised, with girls (M = 2.71, SD = .96) rated as slightly more developed than boys (M = 2.39, SD = .80), consistent with expectations given that girls on average reach puberty earlier than boys. Age and pubertal status were also highly correlated (r = .75).

ANALYSES

In a standard twin model, the variance in the trait is separated into three latent variables representing the amount of variance attributed to additive genetics (*A*), shared environment (*C*) and non-shared environment (*E*; Neale & Maes, 2004). Within twin pair correlations are set such that *A* in MZ twin correlates at 1 and in DZ twins at .5 in accordance with their percentage of shared genes; *C* in all twin pairs correlates at 1 given that they are raised together, and *E* remains uncorrelated and includes measurement error. We fit three behavior genetics models—one each for twin report, parent report, and combined report—as common and specific pathways models, in which three sets of *ACE* factors are fit: one to variance common to anxiety and depression, one to variance unique to anxiety, and one to variance unique to depression (Loehlin, 1996). First, given sex differences in internalizing in adolescence, we fit a model moderated by sex, to determine whether sex differences needed to be evaluated in the subsequent models. A series of moderation models were then fit by age, by pubertal status, and by both simultaneously to assess developmental trends.

Results

All structural equation modeling was completed in Mplus 7.4 (Muthén & Muthén, 2010). All mean scores were log transformed due to positive skew, and were corrected for age, age^2, sex, African American race and Hispanic/Latino ethnicity, and pubertal development. Raw phenotypic correlations are shown in Table 1. The complex sampling option was used to correct for non-independence of twins in phenotypic analyses, and for multiple pairs within a family in behavior genetic analyses. Additionally, triplet sets were weighted due to individuals repeated across pairs.

Table 1. Raw Phenotypic Correlation Matrix

	Sex	Age	Age^2	Hisp/ Lat	AA	Pub Dev	Dep Twin	Anx Twin	Dep Par	Anx Par	Dep Com	Anx Com
Sex	1.00											
Age	.03	1.00										
Age^2	-.02	.19	1.00									
Hisp/ Lat	.03	-.04	-.03	1.00								
AA	-.02	.09	-.01	-.12	1.00							
Pub Dev	-.18	.76	.15	-.02	.05	1.00						
Dep Twin	-.08	.01	.15	.01	.02	.08	1.00					
Anx Twin	-.18	.10	.08	.03	-.03	.14	.56	1.00				
Dep Par	-.04	-.02	.09	.02	.03	.03	.42	.24	1.00			
Anx Par	-.09	-.01	.03	.03	-.04	.03	.30	.38	.60	1.00		
Dep Com	-.08	.003	.15	.02	.03	.08	.90	.51	.76	.50	1.00	
Anx Com	-.17	.07	.07	.04	-.04	.12	.54	.89	.46	.75	.60	1.00

Note:
Sex coded 0 = female, 1 = male; age centered at 13; Hisp/Lat coded 1 = Hispanic/Latino; AA coded 1 = African American.

Results of un-moderated models are reported in the first column of Tables 2 through 4 by reporter. For twin report and combined report, non-shared environment accounted for the most amount of variance specific to anxiety and depression, as well as common to both, and additive genetics contributed significantly to each as well, with negligible influence from the shared environment. Parent report results varied from this slightly: variance specific to anxiety followed this pattern, variance common to both had a predominant additive genetics contribution with significant non-shared environmental influence, and variance specific to depression had a significant contribution for only the non-shared environment, with non-significant additive genetic influence. This base model was then moderated by sex in order to test for differential etiology by sex, with results summarized in column two of Tables 2 through 4. No *ACE* pathways were significantly moderated by sex in any of the three models, indicating genetic and environmental etiology for anxiety and depression does not differ across males and females.

The base model for each reporter was then fit to a series of moderation models, first by age, then by puberty, and then by both age and puberty simultaneously as depicted in Figure 1. Results are summarized by reporter in Table 2 (Twin), Table 3 (Parent), and Table 4 (Combined). For variance specific to anxiety, the only significant moderation was found for increasing additive genetic influence in the combined report with age, however this was not found for puberty, nor when both were entered into the model. No significant moderation was found on any pathway for age or for puberty in twin or parent report models for variance specific to anxiety.

Table 2. Unstandardized Parameter Estimates from Behavior Genetic Models – Twin Report

Variance Common to Anxiety and Depression	No Moderation	Sex Moderation	Age Moderation	Puberty Moderation	Age + Puberty Moderation (Full)
Main effect of A (a_c)	0.42*** (0.06)	0.45***(0.11)	0.29 ` (0.16)	0.30 (0.23)	0.17 (0.20)
Age interaction (a_c')	--	--	0.02 (0.04)	--	0.01 (0.04)
Puberty interaction (a_c'')	--	--	--	0.04 (0.10)	0.07 (0.07)
Sex interaction (a_c''')	--	-0.25(0.68)	--		
Main effect of C (c_c)	0.02 (0.27)	0.04 (0.48)	0.26* (0.10)	0.30 (0.24)	0.16 (0.12)
Age interaction (c_c')	--	--	-0.04* (0.02)	--	-0.06* (0.02)
Puberty interaction (c_c'')	--	--	--	-0.08 (0.08)	0.07 (0.08)
Sex interaction (c_c''')	--	-0.31 (0.23)	--	--	--
Main effect of E (e_c)	0.60***(0.04)	0.61***(0.06)	0.60***(0.04)	0.50***(0.08)	0.55***(0.09)
Age interaction (e_c')	--	--	0.01 (0.01)	--	0.01 (0.02)
Puberty interaction (e_c'')	--	--	--	0.06 (0.05)	0.04 (0.06)
Sex interaction (e_c''')	--	-0.04 (0.09)			
Variance Unique to Anxiety					
Main effect of A (a_a)	0.30***(0.08)	0.29**(0.10)	0.26***(0.08)	0.16 (0.14)	0.27 (0.22)
Age interaction (a_a')	--	--	0.03 ` (0.02)	--	0.04 (0.05)
Puberty interaction (a_a'')	--	--	--	0.09 (0.07)	-0.003 (0.16)
Sex interaction (a_a''')	--	-0.01 (0.17)	--		
Main effect of C (c_a)	0.000 (0.001)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	-0.001 (0.03)
Age interaction (c_a')	--	--	0.000 (0.000)	--	-0.001 (0.03)
Puberty interaction (c_a'')	--	--	--	0.000 (0.000)	0.003 (0.07)
Sex interaction (c_a''')	--	0.000 (0.001)	--	--	--
Main effect of E (e_a)	0.62***(0.04)	0.54***(0.06)	0.61***(0.04)	0.67***(0.07)	0.72***(0.10)
Age interaction (e_a')	--	--	0.002 (0.01)	--	0.02 (0.02)
Puberty interaction (e_a'')	--	--	--	-0.03 (0.04)	-0.07 (0.07)
Sex interaction (e_a''')	--	0.15 ` (0.09)	--	--	--
Variance Unique to Depression					
Main effect of A (a_d)	0.43***(0.06)	0.53***(0.07)	0.38***(0.12)	0.30* (0.14)	0.29 (0.20)
Age interaction (a_d')	--	--	0.02 (0.03)	--	-0.001 (0.03)
Puberty interaction (a_d'')	--	--	--	0.04 (0.11)	0.06 (0.12)
Sex interaction (a_d''')	--	-0.22 ` (0.13)	--		
Main effect of C (c_d)	-0.007 (0.06)	0.000 (0.001)	0.13 (0.17)	0.01 (0.08)	0.06 (0.17)
Age interaction (c_d')	--	--	0.29 (0.16)	--	0.01 (0.03)
Puberty interaction (c_d'')	--	--	--	0.10 (0.10)	0.05 (0.13)
Sex interaction (c_d''')	--	0.000 (0.000)	--		
Main effect of E (e_d)	0.53***(0.05)	0.47***(0.07)	0.53***(0.05)	0.48***(0.09)	0.51***(0.12)
Age interaction (e_d')	--	--	0.01 (0.01)	--	0.01 (0.02)
Puberty interaction (e_d'')	--	--	--	0.04 (0.05)	0.02 (0.07)
Sex interaction (e_d''')	--	0.12 (0.09)	--	--	--

Note:

Age centered at 13 and pubertal status centered at 1. Anxiety and depression scores standardized (M = 0, SD = 1). Age, age-squared, sex, race, and puberty partialled out. SD in parenthesis. $p < 0.1$ = `, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***.

Table 3. Unstandardized Parameter Estimates from Behavior Genetic Models – Parent Report

Variance Common to Anxiety and Depression	No Moderation	Sex Moderation	Age Moderation	Puberty Moderation	Age + Puberty Moderation (Full)
Main effect of A (a_c)	0.56*** (0.05)	0.60***(0.07)	0.42***(0.11)	0.32 (0.28)	0.38 (0.29)
Age interaction (a_c')	--	--	0.08* (0.04)	--	0.06 (0.05)
Puberty interaction (a_c'')	--	--	--	0.14 (0.11)	0.04 (0.13)
Sex interaction (a_c''')	--	-0.09 (0.11)	--	--	--
Main effect of C (c_c)	0.000 (0.000)	0.003 (0.89)	0.15 (0.13)	0.22 (0.48)	0.18 (0.52)
Age interaction (c_c')	--	--	-0.05 (0.03)	--	-0.04 (0.08)
Puberty interaction (c_c'')	--	--	--	-0.06 (0.16)	-0.01 (0.21)
Sex interaction (c_c''')	--	-0.001 (0.24)	--	--	--
Main effect of E (e_c)	0.50***(0.05)	0.52***(0.07)	0.53***(0.05)	0.52***(0.11)	0.48***(0.12)
Age interaction (e_c')	--	--	-0.01 (0.02)	--	-0.02 (0.03)
Puberty interaction (e_c'')	--	--	--	-0.01 (0.06)	0.04 (0.07)
Sex interaction (e_c''')	--	-0.02 (0.11)	--	--	--
Variance Unique to Anxiety					
Main effect of A (a_a)	0.31** (0.08)	0.33** (0.12)	0.32***(0.08)	0.25* (0.10)	0.32 (0.20)
Age interaction (a_a')	--	--	0.02 (0.01)	--	0.02 (0.02)
Puberty interaction (a_a'')	--	--	--	0.04 (0.05)	-0.01 (0.07)
Sex interaction (a_a''')	--	-0.20 (0.38)	--	--	--
Main effect of C (c_a)	0.000 (0.000)	0.004 (0.08)	0.000 (0.000)	0.000 (0.000)	0.09 (0.87)
Age interaction (c_a')	--	--	0.000 (0.000)	--	0.01 (0.10)
Puberty interaction (c_a'')	--	--	--	0.000 (0.000)	-0.03 (0.29)
Sex interaction (c_a''')	--	0.27 ` (0.15)	--		--
Main effect of E (e_a)	0.58***(0.05)	0.58***(0.07)	0.60***(0.04)	0.63***(0.06)	0.51***(0.09)
Age interaction (e_a')	--	--	-0.02 (0.01)	--	-0.03 ` (0.02)
Puberty interaction (e_a'')	--	--	--	-0.03 (0.04)	0.06 (0.06)
Sex interaction (e_a''')	--	0.01 (0.09)	--	--	--
Variance Unique to Depression					
Main effect of A (a_d)	0.13 (0.22)	0.25 (0.16)	0.03 (0.10)	0.32 (0.20)	0.09 (0.19)
Age interaction (a_d')	--	--	-0.09** (0.03)	--	-0.08 ` (0.04)
Puberty interaction (a_d'')	--	--	--	-0.23* (0.11)	-0.04 (0.12)
Sex interaction (a_d''')	--	-0.18 (0.14)	--	--	--
Main effect of C (c_d)	0.000 (0.000)	0.000 (0.001)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Age interaction (c_d')	--	--	0.000 (0.000)	--	0.000 (0.000)
Puberty interaction (c_d'')	--	--	--	0.000 (0.000)	0.000 (0.000)
Sex interaction (c_d''')	--	0.000 (0.000)	--	--	--
Main effect of E (e_d)	0.66***(0.04)	0.61***(0.06)	0.63***(0.04)	0.64***(0.08)	0.62***(0.08)
Age interaction (e_d')	--	--	-0.000 (0.02)	--	-0.001 (0.02)
Puberty interaction (e_d'')	--	--	--	0.002 (0.05)	0.01 (0.05)
Sex interaction (e_d''')	--	0.08 (0.08_	--	--	--

Note:

Age centered at 13 and pubertal status centered at 1. Anxiety and depression scores standardized (M = 0, SD = 1). Age, age-squared, sex, race, and puberty partialled out. SD in parenthesis. $p < 0.1$ = `, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***.

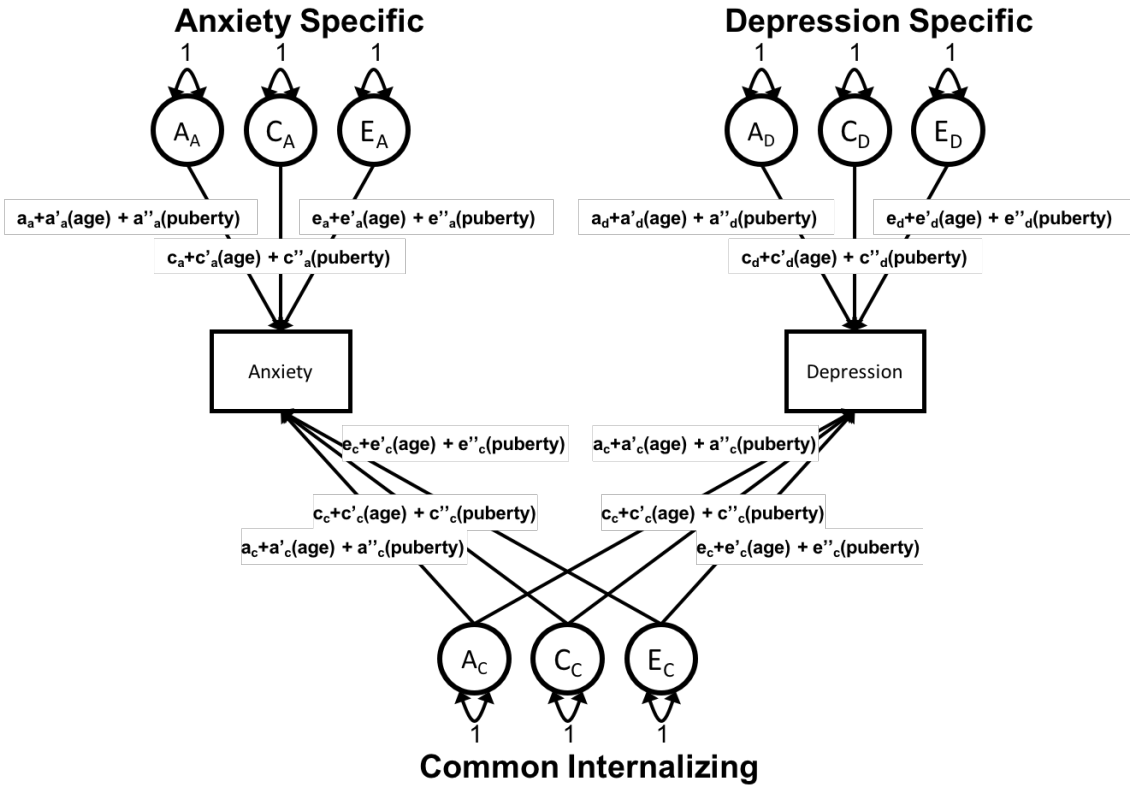
Table 4. Unstandardized Parameter Estimates from Behavior Genetic Models – Twin and Parent Report Combined

Variance Common to Anxiety and Depression	No Moderation	Sex Moderation	Age Moderation	Puberty Moderation	Age + Puberty Moderation (Full)
Main effect of A (a_c)	0.45** (0.16)	0.43 ` (0.26)	0.41***(0.08)	0.47 ` (0.27)	0.29 ` (0.16)
Age interaction (a_c')	--	--	-0.03 (0.07)	--	-0.04 (0.09)
Puberty interaction (a_c'')	--	--	--	-0.003 (0.13)	0.09 (0.08)
Sex interaction (a_c''')	--	-0.54 (0.42)	--	--	--
Main effect of C (c_c)	0.07 (0.77)	0.25 (0.37)	0.001 (0.27)	0.02 (5.87)	0.07 (0.20)
Age interaction (c_c')	--	--	0.07 ` (0.04)	--	-0.06 (0.07)
Puberty interaction (c_c'')	--	--	--	-0.01 (2.12)	0.01 (0.20)
Sex interaction (c_c''')	--	0.13 (0.49)	--	--	--
Main effect of E (e_c)	0.61***(0.05)	0.63***(0.06)	0.60***(0.04)	0.52***(0.09)	0.56***(0.12)
Age interaction (e_c')	--	--	0.02 (0.02)	--	0.01 (0.02)
Puberty interaction (e_c'')	--	--	--	0.05 (0.05)	0.03 (0.07)
Sex interaction (e_c''')	--	-0.03 (0.09)	--	--	--
Variance Unique to Anxiety					
Main effect of A (a_a)	0.31*** (0.08)	0.38** (0.08)	0.27***(0.07)	0.13 (0.15)	0.19 (0.18)
Age interaction (a_a')	--	--	0.04* (0.02)	--	0.02 (0.03)
Puberty interaction (a_a'')	--	--	--	0.11 (0.07)	0.06 (0.11)
Sex interaction (a_a''')	--	-0.23 (0.26)	--	--	--
Main effect of C (c_a)	0.000 (0.000)	0.002 (0.08)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
Age interaction (c_a')	--	--	0.000 (0.000)	--	0.000 (0.000)
Puberty interaction (c_a'')	--	--	--	0.000 (0.000)	0.000 (0.000)
Sex interaction (c_a''')	--	-0.01 (0.39)	--		--
Main effect of E (e_a)	0.57***(0.04)	0.46***(0.06)	0.57***(0.04)	0.64***(0.06)	0.71***(0.09)
Age interaction (e_a')	--	--	0.003 (0.01)	--	0.02 (0.02)
Puberty interaction (e_a'')	--	--	--	-0.04 (0.04)	-0.10 (0.06)
Sex interaction (e_a''')	--	0.21* (0.08)	--	--	--
Variance Unique to Depression					
Main effect of A (a_d)	0.37***(0.06)	0.46***(0.08)	0.37***(0.11)	0.37* (0.15)	0.32 (0.19)
Age interaction (a_d')	--	--	0.01 (0.03)	--	-0.01 (0.04)
Puberty interaction (a_d'')	--	--	--	-0.02 (0.13)	0.02 (0.17)
Sex interaction (a_d''')	--	-0.19 (0.14)	--	--	--
Main effect of C (c_d)	0.000 (0.000)	0.004 (0.03)	-0.05 (0.39)	0.04 (0.08)	0.05 (0.17)
Age interaction (c_d')	--	--	-0.01 (0.07)	--	0.000 (0.03)
Puberty interaction (c_d'')	--	--	--	-0.11 (0.11)	-0.11 (0.15)
Sex interaction (c_d''')	--	-0.003 (0.02)	--	--	--
Main effect of E (e_d)	0.54***(0.04)	0.48***(0.07)	0.52***(0.05)	0.38***(0.11)	0.37**(0.13)
Age interaction (e_d')	--	--	0.02 (0.02)	--	-0.004 (0.02)
Puberty interaction (e_d'')	--	--	--	0.102 (0.06)	0.10 (0.08)
Sex interaction (e_d''')	--	0.11 (0.09)	--	--	--

Note:

Age centered at 13 and pubertal status centered at 1. Anxiety and depression scores standardized (M = 0, SD = 1). Age, age-squared, sex, race, and puberty partialled out. SD in parenthesis. $p < 0.1$ = `, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***.

Figure 1. Common and Specific Pathways Model, Age and Puberty Moderation



For variance specific to depression, significant moderation was found with parent report such that additive genetic influence increased with both age, and with puberty. However, these effects were no longer significant once both were entered into the model, thus we were unable to distinguish whether these were effects specific to chronological age or to pubertal maturation. Moreover, these trends were not seen in twin report, or combined report, in which no moderation by age or puberty was found on any pathway.

For variance common to both anxiety and depression, significant moderation of additive genetic influence increasing with age only was found in parent report, but this was no longer significant once puberty was accounted for simultaneously within the model. Additionally, in the twin report significant decreases of shared environment were found with age, however contributions due to the shared environment were negligible, and this

effect was no longer found once accounting for puberty. No significant moderation was found with the combined report on any pathway for variance common across anxiety and depression.

Taken together, these results reveal a slight trend towards increasing heritability, though highly variable and inconsistent across reporters, and minor enough to be unable to distinguish between an effect of age or of puberty. Overall, differences in genetic and environmental etiology of anxiety and depression were not seen consistently between younger and older twins, or those more or less pubertally developed.

Discussion

Overall, despite some minimal trends towards increasing heritability, developmental etiology remained fairly consistent across childhood and adolescence. The trends that were detected were not consistent across reporters, with twin report yielding only decreases with age in the already minimal shared environmental estimate on common internalizing variance; combined report indicating increases with age in heritability of anxiety; and parent report increases with both age and puberty for heritability of depression, and with age for variance common across internalizing. Furthermore, once age and puberty were entered into the model simultaneously to distinguish moderation effects of each accounting for the other, no developmental trends remained significant. This means we were unable to reliably distinguish between the roles of chronological age or pubertal status in any minimal developmental trends. This indicates that internalizing problems do not appear to be genetically activated by puberty, and in fact show few developmental changes in etiology from childhood through adolescence.

Despite phenotypic increases in internalizing prevalence and the emergence of sex differences in the rates of anxiety and depression around the time of the onset of puberty, genetic risk underlying internalizing stays the same. One potential explanation for this finding could be that the magnitude of genetic and environmental contributions remains relatively stable, though the underlying factors may be shifting. This notion is consistent with evidence that there appears to be attenuation of a genetic factor on internalizing from childhood through adolescence, and increases in additional genetic factors at later developmental stages (Kendler et al., 2008). Additionally, though previous works has shown increases in heritability in anxiety and depression from childhood to adulthood (Bergen et al., 2007), it may be that during this developmental window the magnitude of

these increases is slight, but continues on after adolescence, suggesting that this is largely an effect of chronological age as opposed to pubertal activation.

Previous behavioral genetic work on internalizing disorders has found high variability in estimates from different reporters. Though inconsistent moderation across reporters was detected in the current study, un-moderated estimates were largely consistent across the different reporter models. One notable exception was that parent-report yielded higher heritability common across internalizing, and lower heritability estimates of depression. This could indicate that parents have a decreased ability to report on their children's symptoms of depression relative to anxiety, and instead pick up on general indications of internalizing distress.

It should be noted that the current sample is a community sample, and our measures were indexing anxiety and depression symptoms, not diagnoses. Overall, reports of internalizing problems in our sample were low. This limits the generalizability of our findings to clinical populations. However, subthreshold levels of internalizing problems still contribute to negative life outcomes, and early levels of subthreshold symptoms in adolescence contribute to the development of anxious and affective disorders later in life (Fergusson, Horwood, Ridder, & Beautrais, 2005; Lewinsohn, Solomon, Seeley, & Zeiss, 2000; Preisig, Merikangas, & Angst, 2001).

The current study did not support pubertal activation of genetic risk for internalizing, and found relatively minimal developmental changes in genetic and environmental etiology of anxiety and depression between childhood and adolescence. This leaves open the broader question of what *does* underlie changes in behavioral presentation of internalizing across ages and developmental periods, as well as sex, given that etiology remains fairly consistent across groups. Future work may consider environmental moderators enhancing susceptibility to internalizing disorders, such as

adverse social contexts or increasingly demanding societal roles, that become more prevalent throughout the lifespan.

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