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Maternal Predictors of Anxiety Risk in Young Males with Fragile X

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Abstract

Children with fragile X syndrome (FXS) demonstrate high rates of anxiety disorders, with 65–83% meeting diagnostic criteria. The severity of anxiety symptoms in FXS has been shown to be partially predicted by elevated negative affect across early childhood (Tonnsen, Malone, et al., 2013). This association suggests that biologically-driven vulnerability emerges early in development, as is reported in non-clinical populations. However, anxiety emergence is likely moderated by multifaceted genetic, biological and environmental risk and protective factors. Mothers with the *FMRI* premutation have been shown to exhibit elevated parenting stress and internalizing symptoms, which have each been associated with child behavior problems (Bailey, et al., 2008). Despite these findings, it is unclear whether maternal factors directly relate to anxiety vulnerability in high-risk children with FXS, a question essential to informing targeted, family-sensitive treatment. The present study examines how maternal protective and risk factors relate to child inhibition reflected in (1) child anxiety symptoms, (2) child trajectories of negative affect, and (3) the association between child anxiety and negative affect. Primary predictors include maternal parenting stress, indicators of mental health risk (anxiety and depressive symptoms), and maternal optimism. We also examine genetic correlates in mothers (CGG repeats, activation ratio, mRNA). Our findings suggest that behavioral inhibition in young children with FXS is associated with higher parenting stress and lower optimism, and higher parenting stress is associated with lower maternal X-activation ratio. These findings underscore the need for family-sensitive treatment strategies for anxiety disorders in children with FXS.

Keywords

FMRI; Temperament; Parenting Stress; Behavioral Inhibition

Introduction

Fragile X syndrome (FXS) is the leading known cause of inherited intellectual disability and is highly associated with comorbid disorders such as autism and anxiety (Bailey et al., 2008a; Cordeiro et al., 2011; Kaufmann et al., 2004). The FXS phenotype is caused by an

expansion of >200 CGG trinucleotide repeats in the promoter region of the *FMRI* gene. This mutation results in hypermethylation and reduced mRNA, which in turn silences transcription of fragile X mental retardation protein (FMRP) essential for dendritic translation and synaptic plasticity. The FXS physical phenotype may present as a long, narrow face, protruding ears, and macroorchidism in males. Psychosocial characteristics include high levels of comorbid anxiety disorders, autistic symptoms, and attention problems (Hatton et al., 2009; Roberts et al., 2009c; Hall et al., 2008). Due to the high rates of comorbidities associated with FXS and the known genetic mechanism of the disorder, FXS has received attention in recent years as a potential gateway for understanding complex gene-environment interactions (Restivo et al., 2005), characterizing biomarkers and pathways of neurodevelopmental and psychiatric disorders (Fernández et al., 2013; Hagerman and Hagerman, 2013), and developing targeted treatments for autism and other disorders (Wang et al., 2010).

Anxiety disorders are among the most common and debilitating comorbidities associated with FXS, with up to 86% of males meeting DSM-IV diagnostic criteria using clinical interviews (Cordeiro et al., 2011) and 70% seeking treatment (Bailey et al., 2008a). In non-FXS samples, pediatric anxiety is associated with several established risk factors, with early temperament reported as the best known predictor of anxiety (Hudson and Dodd, 2012; Kagan et al., 1999; Hirshfeld et al., 1992; Biederman et al., 2001). In this work, specific temperament profiles are posited to increase risk for developing anxiety as a constraining, but not determining, factor in the expression of anxiety over time (Fox, Henderson, Marshall, Nichols, & Ghera, 2005). Temperament reflects biologically-mediated individual differences in self-regulation and reactivity and is characterized using three domains of surgency, executive control, and negative affect (Rothbart and Derryberry, 1981). Negative affect, which is comprised of features such as sadness, fear, withdrawal, and anger, is closely related to the construct of behavioral inhibition, a term commonly used to describe a shy, restrained response to unfamiliar situations (Kagan et al., 1989). In the literature on typically developing children, high ratings of negative affect and behavioral inhibition in infancy and toddlerhood are strongly predictive of the emergence and stability of anxiety in later childhood (Brooker et al., 2013; Hudson and Dodd, 2012; Hirshfeld et al., 1992; Biederman et al., 2001). Capitalizing on this association, several recent studies have demonstrated that anxiety symptoms can be reduced or prevented through early treatments targeted towards behaviorally inhibited preschoolers (Bayer et al., 2011b, 2011a; Rapee et al., 2005). These translational efforts demonstrate the utility of characterizing early trajectories of temperament as a means to inform targeted treatment and potential prevention of child anxiety.

Using a temperament framework to investigate the emergence and predictors of anxiety recognizes the complex nature of multiple sources of influence, including environmental, genetic, and developmental factors that interact in a dynamic manner (Brooker et al., 2013). Familial factors are highly implicated in anxiety, with several studies associating maternal anxiety disorders with children's behavioral inhibition and anxiety (Brooker et al., 2013; Aktar et al., 2013; Hudson and Dodd, 2012; Hirshfeld et al., 1992). This association likely arises due to both environmental and genetic factors. Behavioral studies affirm that children

begin to model the anxious responses they observe in parents as early as infancy and toddlerhood (de Rosnay et al., 2006; Gerull and Rapee, 2002; Murray et al., 2008). Maternal anxiety may also result in parental over-involvement and reduced encouragement, which have each been associated with elevated child anxiety symptoms (Murray et al., 2007; Hudson and Dodd, 2012). Genetic factors are also implicated, as both behavioral inhibition (Robinson et al., 1992; Sheese et al., 2009; Holmboe et al., 2011) and anxiety disorders (Stein et al., 1999; Van Houtem et al., 2013) are considered to be highly heritable, and distinct patterns of temperament have been defined in single-gene disorders such as FXS (Bailey et al., 2000; Shanahan et al., 2008).

The association among behavioral inhibition, anxiety, and maternal factors is particularly salient in specific clinical populations, such as families affected by the *FMRI* spectrum of involvement. Elevated anxiety is often reported to be one of the most impairing comorbidities associated with fragile X and is well documented in children and adults with FXS (Cordeiro et al., 2011) and in carrier females (Bailey et al., 2008a; Cordeiro et al., 2011). Although the specific nature of these impairments has not been examined in great detail in FXS, pediatric anxiety is generally associated with impairments across school, social and home domains (Langley et al., 2004). Importantly, atypical negative affect in early childhood has also been associated with anxiety symptom outcomes in FXS (Tonnsen et al., 2013a), consistent with predictive studies in typically developing samples (Brooker et al., 2013; Hudson and Dodd, 2012; Hirshfeld et al., 1992; Biederman et al., 2001). However, little work has been done to characterize other environmental and genetic predictors of anxiety risk and resilience in FXS.

Recent research on individuals with the *FMRI* premutation has enhanced the understanding of familial factors that may relate to anxiety emergence in this population. Mothers of male children with FXS are carriers of *FMRI* gene mutations and may be affected by having the full mutation themselves or by having the *FMRI* premutation. The *FMRI* premutation results from expansions of between 55–200 CGG repeats, which leads to increased mRNA that may yield a toxic effect on cells. Although previously assumed that premutation “carriers” were largely unaffected by the direct genetic mechanisms associated with *FMRI* mutations, it is now evident that a subset of individuals with the *FMRI* premutation experience genetic vulnerability for a variety of conditions, including premature ovarian insufficiency (Allingham-Hawkins et al., 1999); fragile X-associated tremor/ataxia syndrome (FXTAS) (Jacquemont and Hagerman, 2004; Hagerman et al., 2004); and subtle deficits in executive function (Kraan et al., 2013; Cornish et al., 2008) and mathematic reasoning (Lachiewicz et al., 2006). Critical to the present study, women with the *FMRI* premutation also exhibit elevated anxiety and mood disorders (Boyle and Kaufmann, 2010; Kenna et al., 2013; Roberts et al., 2009a), which have been associated with impaired executive function abilities (Kraan et al., 2013). In most cases, these conditions emerge prior to giving birth to a child with FXS (Roberts et al., 2009a), suggesting genetic vulnerability exists independent of environmental factors related to parenting a child with a disability.

Despite these endogenous and exogenous risks, parenting experiences vary across women with the *FMRI* premutation, with the majority of women reporting generally positive adaptation (Bailey et al., 2008b). In one of the few surveys to characterize maternal

adaptation to challenges associated with FXS, Bailey and colleagues (2008) reported that although most women did not score in clinical risk ranges on measures of risk (maternal stress, depressive symptoms, anger, anxiety, low quality of life), over half of the 108 respondents scored in the clinically significant range on at least one measure, most commonly in domains of high stress and low quality of life. Children's problem behaviors, not child intellectual ability, were associated with greater risk for poor maternal adaptation. Thus although most mothers of children with FXS are resilient to parenting challenges, clear vulnerabilities exist that may impact both maternal and child quality of life in a bidirectional manner.

Increased understanding of genetic factors associated with psychiatric comorbidities and psychosocial risk factors in those with a *FMRI* mutation could impact both prevention and treatment efforts for anxiety in FX-associated and non-FX associated samples. In women with the *FMRI* premutation, several studies have reported CGG expansion length associated with both anxiety and depressive symptoms using semi-structured clinical interviews (Kenna et al., 2013; Roberts et al., 2009a) and self-reported symptoms (Johnston and Eliez, 2001; Loesch, et al., 2014; Seltzer et al., 2012), although other studies have found no association (Hessl et al., 2005; Thompson et al., 1994; Franke et al., 1998). These differences may reflect variability in clinical assessment techniques, as well as potential nonlinear associations between CGG repeats and maternal well-being (Loesch, et al., 2014; Seltzer et al., 2012; Roberts et al., 2009a). Activation ratio (AR), which quantifies the proportion of unaffected cells on the X chromosome, has also been associated with neurobiological systems implicated in anxiety. In one of the few studies to examine AR as a risk factor for maternal well-being in women with the *FMRI* premutation, Hartley and colleagues (2012) examined maternal cortisol patterns following days in which their child demonstrated elevated problem behaviors. Women with higher AR demonstrated the expected pattern of elevated morning cortisol, whereas women with lower AR exhibited lower cortisol levels. Among children with FXS, lower AR has also been associated with poorer social responsiveness, social avoidance, and atypically reduced amygdala activity when viewing emotional faces (Kim et al., 2012). Although the specific mechanisms of these associations are not clear, Hessl and colleagues (2005) identified an association between mRNA and anxiety in the *FMRI* premutation only when their sample was restricted to those with AR less than .5, suggesting AR may indirectly influence maternal well-being through other *FMRI*-related mechanisms, such as mRNA. These studies have begun to characterize associations between specific genetic markers and *FMRI*-associated psychiatric symptoms. However, additional work is needed to replicate tentative associations, examine co-associations, and clarify implications for treatment.

The Present Study

Although the presence of anxiety and its genetic correlates have been independently examined in both children with FXS and mothers with the *FMRI* premutation, little work has characterized the intersection of anxiety risk within the parent-child relationship. Given the central role of parents in facilitating anxiety treatment in young children (Luby, 2013; Rapee et al., 2005) and the potential for maternal anxiety levels to perpetuate or buffer symptoms in children (de Rosnay et al., 2006; Gerull and Rapee, 2002; Murray et al., 2008),

understanding these complex, interrelated systems is an essential first step to informing targeted treatment. These associations may be particularly robust in families affected by *FMRI* mutations given the elevated genetic risk for anxiety disorders in children (Bailey et al., 2008a; Cordeiro et al., 2011) and mothers (Boyle and Kaufmann, 2010; Kenna et al., 2013; Roberts et al., 2009a), as well as the presence of elevated parenting stress associated with raising a child with FXS (Wheeler et al., 2007; Bailey et al., 2008b). The current study extends our previous work (Tonnsen et al., 2013) by identifying whether maternal factors are associated with trajectories of negative affect. We also build upon initial findings (Bailey et al., 2008) by examining more specific associations among child anxiety, child temperament, and an expanded set of maternal psychosocial and genetic predictors (e.g. AR, mRNA) than has been previously reported.

The present study examines how parenting stress, maternal protective and risk factors relate to (1) child anxiety symptoms, (2) child trajectories of negative affect, and (3) the association between child anxiety and negative affect. Primary predictors include maternal indicators of parenting stress, mental health risk (anxiety and depressive symptoms) and maternal optimism. We also examine genetic correlates in mothers (AR, mRNA, CGG repeats). We hypothesized that elevated maternal indicators of risk and lower protective factors would predict higher child negative affect and anxiety symptoms. We also hypothesized that higher AR, lower mRNA and lower CGG repeats would be associated with less risk and more resilience in mothers.

Materials and Methods

Participants

Participants included 43 young boys with FXS and their biological mothers who participated in one of two related longitudinal studies spanning 10 years, including an initial study focused on early child development (Bailey et al., 2000; Hatton et al., 2001; Roberts et al., 2009c) and a subsequent study examining family adaptation (Bailey et al., 2008b; Roberts et al., 2009a). The present study included all male participants whose biological mothers completed temperament questionnaires during at least two assessments (mean=3.46, SD=1.32, range 2–7, mean study duration=35 months), resulting in 149 ratings of negative affect across participants. Genetic status was confirmed for children and mothers by reviewing exiting genetic reports as part of study enrollment, and a subset of mothers (n=28) participated in more extensive genotyping (AR, mRNA) through study participation. Table 1 includes child participant characteristics and assessment information.

Child Measures

Negative Affect—The Rothbart Temperament Scales measured negative affect across infancy (Infant Behavior Questionnaire, 3 to 12 months; Rothbart, 1981), toddlerhood (Toddler Behavior Assessment Questionnaire- Revised, 16 to 35 months; (Goldsmith, 1996; Rothbart et al., 2003), and childhood (Children's Behavior Questionnaire (3 to 7 years; Rothbart et al.). The Rothbart Scales demonstrate appropriate convergent validity, discriminant validity, and continuity over time and can be combined across ages using growth curve analyses (Putnam et al., 2008). To isolate negative affect, we created a

composite score by standardizing and averaging the 8 temperament subscales that load on the negative affect domain in children with FXS (Roberts et al., 2013) and in typically-developing children (Rothbart and Ahadi, 2001): approach, shyness, discomfort, fear, anger, sadness, perceptual sensitivity, and soothability.

Anxiety—Child anxiety symptoms were rated by mothers using the DSM-Anxiety raw score from the Child Behavior Checklist 1.5–5 (CBCL; Achenbach, 1991), which consists of ten items that are consistent with DSM-IV anxiety disorder criteria (Achenbach et al., 2003). The CBCL demonstrates high test-retest reliability ($r=.95$), is normed on a large sample, and has been widely-used for children with FXS and other disabilities (Hatton et al., 2002; Kau et al., 2004; Dykens and Kasari, 1997). Compared to the anxious/depressed subscale of the CBCL, the DSM Anxiety subscale has demonstrated higher correspondence with anxiety disorder diagnoses made through structured clinical interviews (Ebesutani et al., 2010). Raw scores on DSM-based symptom scales are converted to T-scores, which fall in the following ranges: average (<66), borderline clinical (66–69), and clinically significant (70+). In our sample, one participant received a T-score in the borderline clinical range and 2 in the clinically significant range, consistent with previously reported ranges in similar samples (Tonnsen et al., 2013a; Wheeler et al., 2007; Dykens and Kasari, 1997). Consistent with publisher recommendations for research, raw scores were used for analyses because T-scores are truncated and do not extend below 50 for the DSM anxiety scale. Because CBCL clinical norms have not been identified specifically for children with FXS, examining developmental changes in raw scores also informs the clinical heterogeneity of symptom severity in this population.

Notably, emerging research suggests anxiety symptoms may aggravate autistic symptoms within FXS (e.g. Talisa, Boyle, Crafa, & Kaufmann, 2014), which may intensify behavioral symptoms in this population. Although our previous work identified a specific association between negative affect and anxiety, not autism, within FXS (Tonnsen et al., 2013), we preliminarily examined associations between anxiety and autism symptoms to ensure autism symptoms did not account for anxiety symptoms in this expanded sample. Controlling for mental and chronological age, correlations between DSM anxiety raw scores and Childhood Autism Rating Scale total scores (Schopler, Reichler, & Renner, 1998) were nonsignificant (Spearman $\rho = .16$, $p = .35$), and no participants who exceeded the clinical anxiety threshold also exceeded the cutoff for autism risk. Thus, autism symptoms likely do not account for anxiety symptoms exhibited in our sample and, given our specific focus on anxiety emergence in FXS, were not examined further in the present study.

Developmental Level—Child developmental level was measured using the age equivalent from the Mullen Scales of Early Learning (MSEL) (Mullen, 1995). The MSEL has excellent interrater reliability ($>.90$), high correspondence the Bayley's Mental Development Index ($r=.70$) and includes five domains, four of which were averaged to construct the age equivalent: fine motor, visual reception, receptive language, expressive language, gross motor (not included). Five children were tested beyond the standard MSEL age limit of 68 months (chronological ages 69–77 months), however their performance-based age equivalents fell within the developmental limits of the MSEL (age equivalents

19–33 months). The developmental level observed in our sample is similar to ranges reported in studies of preschool-aged children with FXS (Owen et al., 2013; Winarni et al., 2012).

Maternal Measures

Descriptive information about maternal risk and protective data is presented in Table 2. We report discrete categories based on normative measures for descriptive purposes; however, all variables were analyzed continuously.

Parenting Stress—Parenting stress was measured using the Parenting Stress Index-Short Form (Abidin, 1986), a 36-item self-report measure of parenting stress as reflected through three subscales: parental distress, difficult child characteristics, and dysfunctional parent-child interaction. Total stress scores above 91 indicate clinically significant levels of stress. Twelve women (31%) exhibited scores in the clinically significant range, consistent with previously reported proportions between 30–42% (Johnston et al., 2003; Bailey et al., 2008b).

Maternal Depressive and Anxiety Symptoms—Maternal depressive symptoms were screened using the Beck Depression Inventory, 2nd Edition (Beck et al., 1996), a 21-item self-report measure commonly used in adolescent and adult populations. The BDI-II yields a total score that falls in one of 4 categories of depressive symptoms: minimal (<14), mild (14–19), moderate (20–28), severe (29–63). Six women (15%) exhibited elevated scores, similar to previously reported proportions of 16–20% on the BDI (Wheeler et al., 2007; Bailey et al., 2008b) but lower than 25–33% reported using other self-report measures (Lachiewicz et al., 2010; Seltzer et al., 2012). Maternal anxiety symptoms were measured using the State Anxiety Scale (Spielberger, 1983). Scores across 20 items are summed and converted into percentile and T-scores, with T-scores above 80% indicating clinical significance. Two women (6%) reported scores in the elevated range, a proportion lower than previously reported proportions between 17–39% using various self-report measures (Bailey et al., 2008b; Lachiewicz et al., 2010).

Maternal optimism—Maternal optimism was measured using the Lifetime Orientation Test, Revised (LOT-R)(Scheier et al., 1994), a brief self-report measure of positive expectations about the future. The LOT-R includes 6 scored items yielding an overall optimism score from 0–24. Scores on protective measures were similar to previously reported values by Bailey et al (2008).

Genetic Markers—We examined three genetic markers in a subset of our sample with available data: AR (n=28), CGG repeats (n=37), and adjusted mRNA (n=26). Participant DNA was analyzed using Southern blot techniques to determine CGG repeats and AR. Activation ratio quantifies the proportion of unaffected cells on the active X chromosome (1.00 = 100% of cells affected). Messenger RNA was analyzed using a 7700 Sequence detector (PE Biosystems, Foster City, CA) and methods described by Tassone (Tassone et al., 2000).

Cognitive Ability and Demographic Factors—As a measure of cognitive ability, mothers also completed the Wechsler Abbreviated Scale of Intelligence (WASI)(Wechsler, 1999). The WASI two-subtest full-scale intelligence quotient was included as a covariate for the present study due to moderate correlations between intelligence quotient and parenting stress (Spearman $\rho = .41$, $p = .01$) and depressive symptoms ($\rho = .31$, $p = .06$). Preliminary analyses indicated non-significant associations between maternal predictors and both family income and number of children with FXS in the home ($p > .10$), therefore these variables were not included in subsequent models.

Procedures

Data were drawn from two highly similar research studies, which each included home-based assessments conducted by a team of two trained research assistants, advanced graduate students or investigators. Rating scales were mailed approximately 2 weeks ahead of the scheduled face-to-face assessments and picked up at the time of the assessment. Given repeated assessments of many measures and the nature of our questions, we included all available negative affect data over time and selected the initial maternal data. We then aligned the child anxiety and developmental level data with the date of the maternal data collection. Although the majority of these data aligned closely, some variability emerged due to rating scales not being completed on time. Thus, we controlled for child age at the time of each measure for all analyses.

Statistical Analyses

Across analyses, primary predictors included maternal parenting stress, indicators of risk (anxiety and depressive symptoms), and optimism. In a subset of our sample with available genetic data, we also examined genetic correlates of predictors in mothers (AR, mRNA, CGG repeats). All analyses were conducted in SAS 9.3 with alpha set at $p = .05$. Based on preliminary graphic and descriptive analyses, the following variables were transformed using the natural log: BDI total score, CGG repeats, mRNA, AR. We also conducted analyses with suspected outliers included and excluded and did not identify any apparent influences of outliers on outcomes. To reduce comparisons, maternal risk factors (depressive and anxiety symptoms) were analyzed as multivariate models given their theoretical alignment.

We first examined predictors of child anxiety symptoms by performing three multiple regression models predicting CBCL DSM-Anxiety raw scores from the following sets of predictors: parenting stress, maternal risk (depression and anxiety), and maternal optimism. All models included child mental age, child chronological age and child and maternal assessments, and maternal cognitive ability as covariates.

Our second phase of analyses used multilevel modeling to examine the association between the same sets of predictors and trajectories of child negative affect across ages. For each model, degrees of freedom were calculated using the between/within method, and variance-covariance matrices were set as unstructured. Age of negative affect measurement was centered at the mean (41.66 months) to facilitate interpretation. We first examined unconditional models of level and linear change in negative affect across age, which

describe overall mean levels and change in negative affect across the sample. We only estimated linear change due to our preliminary examination of empirical growth plots and previous unconditional models conducted in an overlapping sample (Tonnsen et al., 2013a).

We then estimated fixed effects of protective and risk factors on individual growth parameters (level, slope) of child negative affect by including each predictor as a level 2 time-invariant covariate. We first examined the association between anxiety and negative affect in our sample, consistent with (Tonnsen et al., 2013a). We then tested predictors from the three models conducted in the first phase of analyses (parenting stress, risk, optimism). We next added significant predictors from these analyses into the anxiety-negative affect model to test whether predictors moderated the association between child anxiety and negative affect (indicated by an interaction effect). Only significant predictors were examined in anxiety-negative affect models to reduce number of analyses. We calculated pseudo R^2 values to estimate the proportion of variance in the unconditional model explained by each significant conditional model (Singer & Willett, 2003).

To examine the effect of genetic variables on these outcomes, we concluded by conducting multiple regression models examining whether significant predictors identified in our previous two phases of analyses were associated with maternal AR, mRNA, and CGG repeats. We also tested a quadratic model for CGG repeats given previous reports of elevated risk among mid-range repeats (Loesch et al., 2014; Seltzer et al., 2012; Roberts et al., 2009a). To limit multiple comparisons, these secondary analyses were only conducted if maternal factors significantly predicted child anxiety or negative affect in the primary models.

Results

Predictors of Child Anxiety Symptoms

Higher child anxiety symptoms were associated with higher total parenting stress [$F(6,28)=4.06$, model $p=.005$, $\beta = .08$, effect $p < .001$] and marginally associated with higher maternal depressive symptoms [$F(7,19)=2.09$, model $p=.10$, $\beta = 2.30$, effect $p = .01$]. No other main effects were significant.

Predictors of Child Negative Affect

Table 3 contains parameter estimates, variance components, and fit indices of multilevel models of unconditional mean and growth. The average level of negative affect at the mean age of the sample was 3.42, with an average increase of .02 point per month (.24 points per year). Both of these estimates significantly differed from zero. Although our sample produced significant variability in mean levels of negative affect, we did not detect significant variability in slopes ($p = .12$), suggesting we would likely be unable to predict variability in individual change over time. Greater anxiety was associated with higher mean levels of negative affect ($p < .001$) with .09 points negative affect per 1 point anxiety score. Higher mean levels of negative affect were also associated with greater total parenting stress ($p = .002$) and a trend for decreased optimism ($p = .09$). Maternal depressive and anxiety symptoms did not predict negative affect trajectories. The proportion of variance in the

unconditional model explained by each significant model was as follows: child anxiety = 39%; total parenting stress = 36%; and optimism = 42%.

We next examined whether salient predictors from our primary models (parenting stress, optimism) moderated the association between negative affect and anxiety by adding main effects (e.g. parenting stress) and interactions (e.g. parenting stress*child anxiety) to the conditional model of child anxiety predicting negative affect. Interactions were not significant (p 's > .05), indicating the association between child negative affect and anxiety does not vary across levels of parenting stress or optimism.

Association between Parenting Stress and Genetics

Given the association of parenting stress to child anxiety and negative affect, as well as the associations among maternal predictors, child anxiety, and child negative affect, we next used multiple regression to examine whether parenting stress was predicted by maternal genetic factors. Lower AR predicted higher parenting stress [$R^2 = .40$, $F(5, 21) = 2.75$, model $p = .05$, $\beta = -31.82$, effect $p = .008$]. Figure 1 depicts this association. No other associations were significant.

Discussion

Given the high risk for anxiety disorders in children with FXS and the multiple genetic and environmental layers of risk associated with this population, identifying early developmental precursors and predictors of anxiety is essential to informing targeted and effective treatments. In prospective studies, temperament traits of behavioral inhibition and features of negative affect are strong predictors of risk for later anxiety in community samples (Perez-Edgar & Fox, 2005; Schwartz et al., 1999). We previously demonstrated that anxiety symptoms are predicted by trajectories of parent-reported negative affect in young children with FXS (Tonnsen et al., 2013a). In recognition that anxiety risk is multifaceted, the present study sought to identify additional markers of risk and resilience associated with these patterns of negative affect and anxiety in young children with FXS. We also expanded our previous analyses by examining negative affect as a single composite in a larger and older sample (current $n=43$, ages 8–107 months; Tonnsen et al., 2013a $n=25$, ages 8–71 months). Our results indicate that both child anxiety symptoms and levels of negative affect are associated with higher parenting stress, and child negative affect is also marginally related to lower maternal optimism. Higher parenting stress is associated with lower AR in mothers. These findings lend insight into the family experiences and genetic factors that contextualize anxiety emergence in young children with FXS.

Maternal predictors

Across analyses, our results indicate that parenting stress co-occurs with child anxiety symptoms and anxiety precursors, reflected as negative affect, in young boys with FXS. This association between parenting stress and child anxiety is consistent with previous reports that parenting stress strongly correlates with more severe overall problem behavior ratings in children with FXS (Bailey et al., 2008b; Wheeler et al., 2007). Parenting stress is also commonly reported in families of children with autism spectrum disorder (ASD) (Davis

and Carter, 2008), which is co-morbid in 25–60% of children with FXS (Kaufmann et al., 2004; Bailey et al., 2008a). In ASD, higher maternal stress is associated with child difficulties in social relatedness and self-regulation, (Davis and Carter, 2008) features common to the FXS phenotype (Roberts et al., 2007, 2009b; Hall et al., 2009; Tonnsen et al., 2013b). Our data also suggest, however, that early levels of negative affect are associated with parenting stress and low optimism, indicating early temperament may be useful in identifying risk factors within the parent-child relationship. Given 30–50% of mothers of children with FXS report stress levels in the clinically significant range (Bailey et al., 2008b; Wheeler et al., 2007), identifying early precursors of risk may inform service coordination protocols for affected families.

Although we identified an association between parenting stress and child symptoms, we did not find the anticipated association between maternal mental health risk (depression and anxiety) and child negative affect or child anxiety. This finding was somewhat surprising given that Bailey and colleagues (2008b) identified associations between overall child problem behaviors and both maternal depression and anxiety in an overlapping sample of families. Three differences between studies may account for these distinct findings. First, whereas Bailey and colleagues focused on the broader Total Problem Behaviors score on the CBCL, we exclusively examined the DSM Anxiety subscale given our specific focus on anxiety risk. Thus child problem behaviors studied by Bailey and colleagues may broadly relate to maternal mental health, whereas child anxiety may associate more closely with situational stressors associated with parenthood. Second, our sample of mothers reported lower incidences of anxiety (6%) than previously reported by Bailey and others (17–39%) (Bailey et al., 2008b; Lachiewicz et al., 2010). Although levels of depression were similar to some previous reports (15% versus 16–20%) (Wheeler et al., 2007; Bailey et al., 2008b), prevalence rates were lower than those reported by other groups using self-report measures (25–33%) (Seltzer et al., 2012; Lachiewicz et al., 2010). Additionally, some mental health risks associated with the FMR1 premutation increase with age (Tassone et al., 2012), thus it is possible that restricted variability or less severe presentation in mental health reports by mothers in our sample precluded examination of child correlates. Third, although a subset of families participated in both our and Bailey and colleagues' studies, our sample was smaller (n=43 versus n=108) and the mean child age in our sample is younger (58 months versus 72 months) due to our focus on temperament during early childhood. The age differences between studies is particularly important because anxiety symptoms often begin to exceed clinical thresholds during early childhood or adolescence (Kessler et al., 2007) and only three children in our study received elevated anxiety scores on the CBCL based on norms derived in non-FXS samples. Thus, associations between child anxiety and maternal factors may become more robust as children mature beyond the ages represented in the present study, an important developmental consideration in need of empirical study.

Although additional work is needed to clarify the longitudinal emergence of anxiety in FXS, our results suggest that even at young ages, specific aspects of maternal well-being – maternal parenting stress in particular – associate with child anxiety symptoms. Thus, consistent with evidence that family involvement strengthens anxiety treatment outcomes in children without FXS (Barrett et al., 1996), supporting maternal well-being is likely essential to ensuring effective anxiety treatment for children with FXS, as well. In favor of

this possibility, Greenberg and colleagues (2012) found that fewer child problem behaviors were associated with greater maternal warmth and positivity and lower maternal criticism in families affected by FXS.

Despite the increasing body of literature characterizing psychosocial risks in children with FXS, there is a paucity of syndrome-specific treatments necessary to support families affected by *FMR1* mutations due, in part, to challenges implementing treatment to a population with a relatively low prevalence (~1:4500). Emerging evidence in non-FXS samples suggests it is possible to prevent the manifestation of anxiety in behaviorally inhibited children through parent-mediated intervention (LaFreniere and Capuano, 1997; Rapee et al., 2005). For example, Rapee and colleagues (2005) implemented a 6-session parent intervention for 146 children identified as at-risk due to elevated behavioral inhibition. Treatment was associated with fewer anxiety disorders 1 year after the intervention, suggesting that parents can successfully reduce anxiety symptoms in children, given appropriate support. However, studies have also demonstrated that maternal anxiety, negative affect, and low emotional warmth predict poorer child responses to anxiety treatments (Hudson et al., 2013; Festen et al., 2013). Parenting stress may also hinder treatments contingent on parent-child interactions, as higher levels of parenting stress are associated with fewer parent-child interactions in families affected by FXS (Wheeler et al., 2007). Thus, although emerging work suggests anxiety symptoms may be prevented in behaviorally inhibited preschoolers, our findings support the critical role of maternal well-being in successful prevention efforts in FXS, as well.

Genetic markers

We also examined and found some potentially important genetic risk factors. Specifically, women with lower AR presented greater vulnerability for higher parenting stress. In the only previous study to report significant psychosocial correlates of AR in the *FMR1* premutation, Hartley and colleagues (2012) found that following days in which children with FXS exhibited high behavior problems, women with lower AR demonstrated a lower cortisol response than women with higher AR. The authors note that similar patterns have been identified in other clinical samples with elevated stress, such as adults with PTSD and signs of work overload. Similarly, Hessel and colleagues (2005) identified an association between mRNA and anxiety in women with the *FMR1* premutation with AR less than .5, suggesting AR may indirectly influence maternal well-being through mRNA production. Although we did not find a similar association between mRNA and maternal factors in our sample, perhaps due to the small proportion of women in our sample with AR $\leq .5$ ($n=5$ of 28), our finding that lower AR is associated with higher parenting stress indicates that AR may present a salient clinical biomarker of risk in women with the *FMR1* premutation. However, additional work is needed to examine additive and interactive effects among multiple genetic markers (AR, CGG repeats, mRNA) and stress-related environmental factors to both clarify these processes and inform intervention.

Other genetic markers we investigated were not associated with maternal or child factors. We did not identify an association between CGG repeats and depression, anxiety, or optimism, consistent with Bailey and colleagues' (2008) report in an overlapping, larger

sample. Other studies investigating this association have produced inconsistent results. Some have reported longer (Kenna et al., 2013) or non-linear (Loesch et al., 2014; Roberts et al., 2009a; Seltzer et al., 2012) CGG repeat length associated with anxiety and depression using structured clinical interviews and self-reported symptoms (Johnston and Eliez, 2001). Other studies have not identified an association (Bailey et al., 2008a; Hessel et al., 2005; Thompson et al., 1994; Franke et al., 1998). Importantly, women in our sample also reported lower rates of anxiety risk (6% in clinical range versus 17–39% in Bailey et al., 2008a; Lachiewicz et al., 2010) and depression risk (15% in clinical range versus 25–33% in Lachiewicz et al., 2010; Seltzer et al., 2012), thus, the association between mental health and CGG repeats may reflect variability in the severity of clinical symptoms within samples.

Limitations

Despite our novel findings related to anxiety precursors and maternal risk, our findings are limited in several ways. First, we examined associations among longitudinal negative affect trajectories and maternal predictors sampled from a single time point. To inform the direction of effects among variables, future studies may tease apart the longitudinal interrelationships among maternal well-being and associated child factors, integrating an expanded set of child variables including autistic features. Future studies with larger samples may also examine potential mediators and moderators that sustain the relationship between AR and parenting stress and optimism, including child factors and life events. Although our brief self-report measures appear to be sensitive to maternal functioning, additional work is needed to examine maternal psychopathology with greater specificity using structured diagnostic interviews and experimental tasks. Similarly, we measured child anxiety symptoms using a parent-report screening measure. Future studies may instead employ clinically-verified diagnostic interviews or experimental tasks to better tease apart the specific associations between child anxiety and developmental factors. We also restricted our analyses to maternal-child effects given our interest in biobehavioral associations related to the spectrum of *FMRI* mutations. Future studies may also examine the effects of protective and risk factors exhibited by fathers and other family members on child anxiety development.

Conclusion

Given the high prevalence of anxiety in FX-associated conditions and its debilitating effects on the child and family system, characterizing the multifaceted environmental and genetic risk factors associated with anxiety emergence is critical to facilitate identification and early treatment. Emerging studies in community samples suggest that interventions can reduce or prevent anxiety symptom emergence in children at high risk, particularly those with a temperament profile characterized with negative affect and behavioral inhibition (Rapee et al., 2005; LaFreniere and Capuano, 1997). Our findings suggest that negative affect and anxiety symptoms in young children with FXS are closely associated with parenting stress and lower optimism, with genetics implicated as a mechanistic factor for specific stress-related associations.

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Table 1

Descriptive Statistics – Child Data

	<i>N</i>	<i>M</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>
Temperament Assessments					
Age across assessments	149	41.67	21.61	8.44	107.30
Number of assessments	149	3.46	1.32	2	7
Chronological Age at first asmt	43	40.31	12.59	9.03	79.21
Mental Age					
MSEL Mental Age	43	26.64	9.84	10.75	46.75
Chronological Age at MSEL	43	55.60	14.90	18	77
Anxiety Symptoms					
CBCL DSM-Anxiety raw score	37	4.38	2.85	0	13
CBCL DSM-Anxiety <i>t</i> -score	37	56.49	7.23	50	81
Age at CBCL	37	57.57	12.13	22	71
Child FMRP	16	11.69	7.88	2	28

Note. Age reported in months. MSEL = Mullen Scales of Early Learning. CBCL = Childhood Behavior Checklist 1.5–5. FMRP = Fragile X Mental Retardation Protein.

Table 2

Descriptive Statistics – Maternal Data

	<i>N</i>	<i>M</i>	<i>SD</i>	Min	Max
Maternal Predictors					
Total Parenting Stress (PSI)	39	84.36	20.64	44	132
Total Depressive Symptoms (BDI)	39	7.33	8.07	0	39
State Anxiety (STAX) Raw Score	32	15.31	3.57	10	25
State Anxiety (STAX) T Score	32	45.31	7.90	34	66
Total Optimism Score (LOT-R)	40	15.48	4.43	3	23
Maternal Genetic Markers					
Activation Ratio	28	0.68	0.19	0.2	0.94
Adjusted mRNA	26	6.05	4.20	2.03	19.26
CGG Repeats	37	91.43	19.99	68	163
Cognitive Ability (WASI)	40	107.23	15.01	61	131

Note. PSI = Parenting Stress Index. LOT-R = Lifetime Orientation Test-Revised. BDI = Beck Depression Inventory. STAX = State Trait Anxiety Scale. WASI = Wechsler Abbreviated Scale of Intelligence. Adjusted mRNA accounts for activation ratio using methods outlined by entirker et al., 2009.

Table 3

Negative Affect Multilevel Models

Model	Est.	SE	df	t	Model p
Unconditional Models					
Intercept	3.42	.07	42	47.58	<.001
Slope	0.02	.00	105	7.72	<.001
Child Anxiety					
Intercept	.09	.03	30	3.04	.005
Slope	0	0	93	-0.72	0.47
Parenting Stress					
Intercept	.008	.003	33	2.04	.05
Slope	0	0	97	0.43	.67
Maternal Depression					
Intercept	.17	.12	20	1.35	.19
Slope	.004	.005	62	.76	.45
Maternal Anxiety					
Intercept	.02	.03	20	0.94	.35
Slope	0	0	62	-0.79	.43
Maternal Optimism					
Intercept	-.03	.02	34	-1.75	.09
Slope	0	0	99	-1.00	.32

Note. Maternal depression and anxiety were analyzed in the same model. Covariates included child mental and chronological age and maternal cognitive abilities.