J Neurodevelop Disord (2009) 1:283–291
DOI 10.1007/s11689-009-9028-5

Autistic behavior in boys with fragile X syndrome: social approach and HPA-axis dysfunction

Jane E. Roberts · Megan A. Clarke · Kaitlyn Alcorn · John C. Carter · Anna C. J. Long · Walter E. Kaufmann

Received: 12 February 2009 / Accepted: 26 July 2009 / Published online: 19 August 2009

Abstract The primary goal of this study was to examine environmental and neuroendocrine factors that convey increased risk for elevated autistic behavior in boys with Fragile X syndrome (FXS). This study involves three related analyses: (1) examination of multiple dimensions of social approach behaviors and how they vary over time, (2) investigation of mean levels and modulation of salivary cortisol levels in response to social interaction, and (3) examination of the relationship of social approach and autistic behaviors to salivary cortisol. Poor social approach and elevated baseline and regulation cortisol are discernible traits that distinguish boys with FXS and ASD from boys with FXS only and from typically developing boys. In addition, blunted cortisol change is associated with increased severity of autistic behaviors only within the FXS and ASD group. Boys with FXS and ASD have distinct behavioral and neuroendocrine profiles that differentiate them from those with FXS alone and typically developing boys.

Keywords Fragile X · Social anxiety · Autism · Social approach · Cortisol

J. E. Roberts
Department of Psychology,
Barnwell College University of South Carolina,
Columbia, SC, USA

M. A. Clarke · K. Alcorn · J. C. Carter · W. E. Kaufmann (✉)
Center for Genetic Disorders of Cognition & Behavior,
Kennedy Krieger Institute,
716 N. Broadway,
Baltimore, MD 21205, USA
e-mail: Kaufmann@KennedyKrieger.org

A. C. J. Long
Frank Porter Graham Child Development Institute,
University of North Carolina,
Chapel Hill, NC, USA

W. E. Kaufmann
Departments of Pathology, Neurology, Pediatrics,
Psychiatry & Behavioral Sciences, and Radiology & Radiological Science, The Johns Hopkins University School of Medicine,
Baltimore, MD, USA

Introduction

Fragile X syndrome (FXS) is the most prevalent form of inherited intellectual disability, affecting approximately 1:4,000 males and 1:8,000 females [1]. This single gene disorder is linked to the expansion of a CGG polymorphism in the (5′UTR) regulatory region of the *FMR1* gene. Individuals with the full mutation (>200 vs. a normal range of 5–40 CGG repeats) typically have hypermethylation of the *FMR1*'s promoter, resulting in transcriptional silencing of the gene and virtual absence of the Fragile X Mental Retardation Protein (FMRP) [2]. FMRP is a widely expressed RNA-binding protein that regulates protein synthesis at synaptic sites [3, 4]. Magnitude of FMRP deficit correlates with overall severity of physical and neurobehavioral phenotype [5–7], but not consistently with selective behavioral abnormalities such as autistic behavior [8, 9]. Most males with FXS tend to have mild to moderate intellectual disability, characterized by variable cognitive and language impairments, and associated neurobehavioral problems [2, 10].

Among the most frequent and severe behavioral abnormalities present throughout the spectrum of impairment in FXS are disturbances in social interaction including extreme shyness, social withdrawal, social avoidance, social anxiety, and autism spectrum disorder (ASD) [11–14]. Of these social abnormalities, only ASD has been systematically studied in FXS. There is general agreement

that a large proportion of males with FXS meet DSM-IV criteria for autistic disorder. Recent studies using the Autism Diagnostic Observation Scale-Generic (ADOS-G) [15] or the Autism Diagnostic Interview–Revised [16] reflect an increase in prevalence estimates with 25% to 50% of males with FXS now described as meeting criteria for autism and 67% to 74% meeting criteria for the ASD [9, 13, 17–19].

Several studies have focused on different aspects of ASD in FXS, demonstrating in relationship with non-autistic FXS counterparts: lower IQ [12, 20–22], poorer adaptive behavior skills [12, 23], and greater receptive language delays [17, 22]. Recently, there has been increased interest in studying a continuum of autistic behaviors and in identifying specific behaviors that differentiate autism in FXS from the broad phenotype or other related disorders such as social anxiety. This work has shown that poor eye contact and social avoidance in initial social encounters, particularly in less familiar settings [24]; self-injurious and compulsive behaviors [19]; and low adaptability [25] are pervasive in FXS and are not specific to those who have co-morbid ASD diagnoses. In contrast, poor eye contact and social avoidance after sustained interaction with people [24], social indifference and passive social avoidance [14, 26], and severe deficits in non-verbal social behaviors and withdrawn behavior [12, 13, 26, 27] appear to be unique to individuals with FXS and ASD.

Abnormal activation of the hypothalamic-pituitary-adrenal (HPA) axis is cited as one of the primary correlates of social and emotional dysfunction in FXS [28]. The HPA axis responds to and regulates stress by secretion of corticotropin releasing hormone, which stimulates the secretion of ACTH by the pituitary gland, leading to release of cortisol by the adrenal glands [29]. Initial studies reported elevated diurnal salivary cortisol levels and hyper-reactivity in response to challenge in males with FXS compared to their unaffected siblings [30, 31]. Subsequent studies focused on more discrete time points related to social challenge demonstrated elevated cortisol for pre-challenge conditions, but similar cortisol levels for post-challenge and reactivity (subtracting cortisol level prior to challenge from cortisol during challenge) in males with FXS compared to unaffected siblings [27]. Inconsistent relationships between cortisol and problem behavior in FXS have been reported. In an early study, a relationship between a diurnal cortisol composite and the Total score and Withdrawn subscale of the Child Behavior Checklist (CBCL) [32] were reported [31]. However, subsequent work using a more discrete measure of cortisol collected during a pre-challenge condition was not related to any CBCL composite or subscales [27]. Consistent across multiple studies is a relationship between elevated pre-challenge cortisol levels and autistic behaviors including increased gaze avoidance during a social challenge [19, 27] and higher total scores on the ADOS-G [19]. Additionally, one study reported a relationship between suppressed cortisol reactivity to a social challenge and increased gaze avoidance [27].

Taken together, this work indicates that environmental factors including setting, length of interaction, and stress associated with social interaction or challenge is associated with autistic behavior in FXS and that abnormal HPA function may contribute to the presence and severity of autistic behavior in FXS. Identification of the underlying mechanisms and environmental presses that convey increased risk for autistic behavior in FXS is critical to facilitate accurate diagnoses as well as to develop interventions including pharmacological agents, behavioral treatments, and environmental modification(s).

To date, no study has examined the social approach behaviors in males with FXS compared to a non-familial typically developing comparison group using a dynamic observational measure that includes multiple scales over time. Also, only one study has been published that reported pre-challenge, challenge, and reactivity conditions to examine discrete cortisol levels across groups. However, their measure was taken mid-day after the participants had undergone various assessments that morning. Therefore, while it was taken just before their social challenge, the authors note that cortisol elevations that occurred earlier in the day could have limited the pre-challenge cortisol response [27]. The overarching purpose of the present study is to examine environmental and neuroendocrine determinants of autistic behavior in boys with FXS. This is accomplished by evaluating multiple indicators of social approach and cortisol and their inter-relationship over time in boys with FXS (with and without ASD), compared to a community sample of typically developing boys. We have applied a set of three analyses that focus on different components of the relationship of environmental and neuroendocrine predictors of ASD in boys with FXS compared to a typically-developing group of boys. The first analysis examines multiple dimensions of social approach behaviors and how they vary over time. The second analysis investigates mean levels and modulation of salivary cortisol levels in response to social interaction. The third analysis examines the relationship of social approach and autistic behaviors to salivary cortisol. Data for these analyses were drawn from multiple independent, yet related, studies of development in FXS that used a common set of measures across participants. Combining data across multiple studies increased the sample size, allowing for more confidence in the findings.

We hypothesize that boys with FXS + ASD will be distinguished from boys with FXS-only (without ASD) and the typically developing group by their chronic social avoidance, elevated cortisol, and strong relationship between autistic behaviors and cortisol.

Method

Participants

Data for these analyses were drawn from the Carolina Fragile X Project, a series of longitudinal studies that recruited children across the United States through a FXS parent list serve, a University of North Carolina (UNC) Fragile X research registry, FXS family support groups, and brochures sent to FXS clinics and research projects. All males with FXS had the *FMR1* full mutation based on standard DNA testing. Females were excluded due to statistical power considerations and limited control group matches. Descriptive information including gender, age and adaptive behavior was available for all participants. Groups were gender matched and age was included as a co-variate when groups varied on age. Boys with FXS were subdivided into two groups based on the level of autistic behavior as detailed below. Table 1 displays a descriptive profile of the three study groups across the three analyses.

Measures

Autistic behavior

The CARS is a widely used and reliable measure of autistic behavior in children, with alpha coefficients often at or exceeding 0.85 [33–36], and a test-retest reliability of 0.88 [37]. The CARS consists of 15 items characteristic of autistic behavior and a total score. Examiners rate each item between 1 (typical) to 4 (severely abnormal) and the continuum for the total scores ranges from not autistic (15–29.5), mildly/moderately autistic (30–36), to severely autistic (≥37). As in previous studies, the CARS was used to describe autistic behavior and to categorize participants as either FXS-only or FXS + ASD, rather than to diagnose autism in FXS [21,

Table 1 Characteristics of the participants across analyses

Analysis Series	Participant Groups		
Analysis 1: SAS Profiles	Typically Developing Group	FXS-only	FXS + ASD
	(*n*=21)	(*n*=33)	(*n*=18)
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Chronological Age (Years)[bc]	4.05±1.95	3.99±2.25	8.13±3.55
Vineland Adaptive Behavior Composite[abc]	98.7±10.7	62.7±12.1	41.1±13.3
Childhood Autism Rating Scale Total[c]	N/A	24.8±3.2	34.0±3.7
Analysis 2: Cortisol	(*n*=63)	(*n*=53)	(*n*=11)
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Chronological Age (Years)[ab]	4.88±1.39	7.5±4.06	7.46±4.16
Vineland Adaptive Behavior Composite[abc]	98.7±10.7	55.7±16.0	43.5±13.1
Childhood Autism Rating Scale Total[c]	N/A	24.8±3.1	33.7±3.3
Analysis 3: Behavior Ratings and Cortisol			
Cortisol-SAS	(*n*=21)	(*n*=34)	(*n*=9)
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Chronological Age (Years)[bc]	4.05±1.95	3.99±2.25	8.13±3.55
Vineland Adaptive Behavior Composite[abc]	98.7±10.7	62.7±12.1	41.1±13.3
Childhood Autism Rating Scale Total [c]	N/A	24.8±3.2	34.0±3.7
Cortisol-CARS	(N/A)	(n=40)	(n=10)
	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)
Chronological Age (Years)	N/A	8.03±3.95	7.9±4.1
Vineland Adaptive Behavior Composite[c]	N/A	55.1±13.5	41.0±13.1
Childhood Autism Rating Scale Total[c]	N/A	24.6±3.1	32.7±2.0

[a] Significant difference between Typically Developing Group and FXS-only

[b] Significant difference between Typically Developing Group and FXS + ASD

[c] Significant difference between FXS-only and FXS + ASD

24, 38]. Children with a score of ≥30 were characterized as being on the autism spectrum (ASD) and thus classified as FXS + ASD while those with a score <30 were classified as FXS-only. Each child was evaluated per administration guidelines [33], and ratings were based on direct observation, parent interview, and review of the parent rating scales, after which, the examiners came to a consensus over the numerical ratings [8, 21]. CARS scores are not available for the typically developing group.

Social approach

The Social Approach Scale (SAS) is a time-sensitive experimental measure of multiple forms of social approach behavior. As reported, our group modified the original SAS [39] to include social approach behaviors characteristic of FXS: physical movement (MO; e.g., walks away), facial expression (FA; e.g., fearful facial expression), and eye contact (EC; e.g. avoids eye contact) [24]. Higher scores reflect more social avoidance.

Over the course of an assessment, examiners assign scores at multiple time points to compare initial and sustained social interactions. Data for this study reflect two time points: "initial" ratings, taken within the first minute of the assessment, and "familiar" (with the assessor) ratings, taken in the last hour of the assessment. The average elapsed time between the initial and regulation SAS ratings was 3 h (x=3.00; s.d. = .98). Two examiners were present for each assessment with one primarily responsible for assessing the child and the other primarily responsible for logistics and interactions with the child and mother. Thus, each examiner experienced different types of interactions with the children and for varied amounts of time. The SAS was therefore rated by a consensus across the two examiners to take these factors into account [24].

In our previous work using the SAS, we identified relationships between social approach, social withdrawal, and autistic features and established the SAS as a sensitive and, in this way, valid measure of social approach behaviors in children with FXS [24].

Cortisol

To study potential HPA-axis abnormalities in FXS and its relationship with social approach behavior, two samples of salivary cortisol were taken for each participant. The first cortisol level, labeled "baseline cortisol", was taken within 15 min of the onset of the assessment to reflect pre-evaluation cortisol levels. The second cortisol level, which we refer to as "regulation cortisol", was taken at the end of the SAS evaluation. In addition, we calculated a difference score based on the change in cortisol (regulation cortisol minus baseline cortisol), which we refer to as "delta cortisol". This measurement is meant to serve as an index of cortisol reactivity and to aid in the interpretation of the participants' physiological response to the social challenge. Recent findings have linked lower cortisol reactivity to autistic behavior in FXS so we were interested in examining these relationships in our sample [27].

The cortisol sample was taken via an oral cotton swab, which soaked in the participant's mouth for 1–2 min. To prevent contamination of saliva samples, participants were asked to avoid consumption of products containing citric acid and dairy products for at least 60 min prior to sampling [40, 41]. The precise time of cortisol collection was recorded to account for the diurnal variation in cortisol levels. The saliva was processed using the Salimetrics' Salivary Cortisol Enzyme Immunoassay kit (EIA). The correlation between serum and saliva cortisol using the Salimetrics EIA is highly significant (r=0.91, p<0.0001) (Salimetrics LLC, 2005). The mean inter-assay coefficient of variation was 7.70% (5.40%–8.11%) and the mean intra-assay coefficient of variation was 7.16% (6.88%–7.12%). Each sample was assayed in duplicate, and duplicate correlations were >.95. Cortisol levels were measures in micrograms/deciliter.

Adaptive behavior

The Vineland Adaptive Behavior Scale (VABS) is widely used to measure adaptive behavior skills of individuals from birth to 90 years of age [42]. The Adaptive Behavior Composite (ABC), which integrates VABS' four domains, was calculated based on an interview with the parent(s) of the participant. The VABS has acceptable content and criterion-related validity and a test-retest reliability of r=0.88. In our study, we used the VABS as a descriptive measure of overall adaptive ability for the FXS and typical cohorts.

Data analysis

The primary goal of this study is to examine environmental and neuroendocrine determinants of autistic behavior in young boys with FXS. To accomplish this, we conducted a series of analyses of covariance (ANCOVA) to examine SAS scale scores (i.e., physical movement, facial expression, and eye contact) at initial and familiar assessment intervals as predicted by group while controlling for age. We followed up with a regression of CARS total score as a continuous variable within the group with FXS. Next, we ran ANCOVAs to examine cortisol levels at initial and regulation assessment intervals as predicted by group and age and followed these with a regression of CARS total score within the group with FXS. Finally, cortisol levels

and their correlations with SAS scale scores (for all 3 groups) and CARS total scores (for the 2 groups with FXS) were analyzed using multiple regression models with age controlled for. Adaptive behavior was also used as a covariate in most models. Stringent post-hoc analyses were run in order to minimize the effects of variance heterogeneity, non-Gaussian distribution, and unequal N values [43]. The cortisol data were skewed so they were transformed by taking the log10 of the raw data to make it more amenable to comparative analyses. Hierarchical Bonferroni corrections were used for adjusting for multiple comparisons. Data reported in the Results section has been covaried and/or corrected, unless otherwise specified.

Results

SAS profiles

ANCOVA results indicate that the typically developing group had more approaching physical movement, facial expression, and eye contact than both groups of boys with FXS (with and without ASD) for both initial and familiar social interactions with the exception of physical approach during familiar interactions that was not different from boys with FXS-only (see Fig. 1 and Table 2). Interestingly, the *initial* social approach of the two groups of boys with FXS was indistinguishable across all three SAS scales; however, the boys with FXS + ASD showed less social approach than boys with FXS without ASD during social interactions with *familiar* individuals across all three SAS scales. Regression analyses of SAS measures versus CARS total score within the FXS group (not separated for ASD) supported the ANCOVA findings with no relationship between CARS and *initial* SAS scales; yet, a strong relationship between all three *familiar* SAS scales and CARS total score ($p \leq .005$). The relationship between CARS and familiar physical movement was also significant for the FXS + ASD subgroup ($p < 0.05$).

Cortisol

ANCOVA results show that both baseline (pre-assessment) and regulation (post-assessment) cortisol were higher in boys with FXS + ASD compared to both typically developing boys and boys with FXS without autism, which were not different from each other (see Fig. 2). There were no differences in delta (change) cortisol among any of the three groups.

Behavior ratings and cortisol

SAS and Cortisol. Multiple regression analysis results demonstrate that initial social approach (facial expression and eye contact) had a significant direct relationship to regulation and delta cortisol, while familiar social approach (facial expression and eye contact) had a direct relationship to baseline cortisol in the typically developing group (see Table 3). There was also a significant inverse relationship between initial physical movement and regulation cortisol in the FXS + ASD group ($p = 0.028$).

CARS and Cortisol. The relationship between the CARS and cortisol was examined in the entire group of boys with FXS (not separated for ASD) then in the two groups with FXS (with and without ASD). Regression analyses of cortisol to CARS total score within the entire FXS group revealed no relationship of CARS to cortisol. In contrast, a multiple regression analysis demonstrated an inverse relationship between CARS scores and delta cortisol in the FXS + ASD group ($p = 0.008$). No relationship between

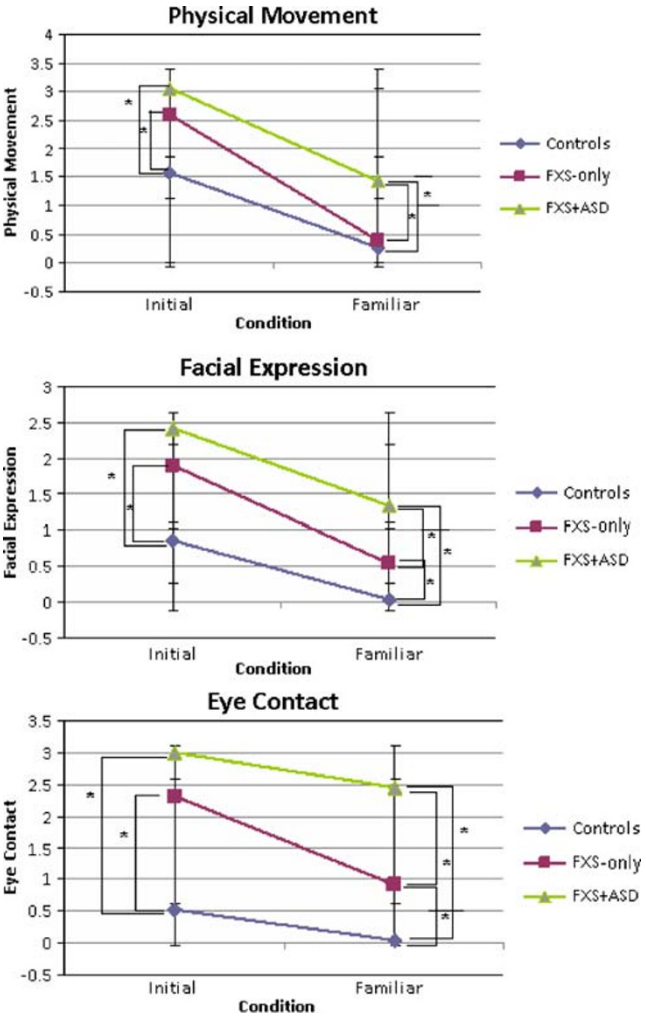

Fig. 1 SAS profiles in FXS. The two FXS groups, FXS-only and FXS + ASD, showed in general less approach than controls in both initial and familiar social encounters. However, only the FXS + ASD group differentiated from FXS-only and the typically developing group in all three scales during familiar social interactions (i.e., persistence of avoidant behaviors)

Table 2 SAS Profile ANCOVA Results

	Typically Developing Group vs. FXS-only (p-value, F-value)	Typically Developing Group vs. FXS + ASD (p-value, F-value)	FXS-only vs. FXS + ASD (p-value, F-value)
Initial Approach			
Physical Movement	***p*=0.0012, F=11.987**	***p*<0.0001, F=12.590**	p=0.1929, F=0.371
Facial Expression	***p*=0.0008, F=12.792**	***p*<0.0001, F=26.616**	p=0.1583, F=1.563
Eye Contact	***p*<0.0001, F=24.122**	***p*<0.0001, F=62.756**	p=0.1285, F=1.612
Familiar Approach			
Physical Movement	p=0.5093, F=0.472	***p*=0.0003, F=1.969**	***p*=0.0002, F=2.867**
Facial Expression	***p*=0.0018, F=10.808**	***p*<0.0001, F=22.628**	***p*=0.0019, F=8.935**
Eye Contact	***p*=0.0005, F=13.982**	***p*<0.0001, F=54.041**	***p*<0.0001, F=12.060**

Age was used as a co-variate in all analyses due to the fact that the FXS + ASD group was substantially older than both the typically developing group and FXS-only group

the CARS score and any of the other cortisol measures in the boys with FXS-only was found. These analyses were only conducted with the boys with FXS as the CARS was not available for the typically developing group

Discussion

The primary goal of this study was to examine environmental and neuroendocrine factors that convey increased risk for elevated autistic behavior in boys with FXS. Towards this end, we employed a dynamic multidimensional observational scale of social approach behavior and scales of autistic behavior in boys with FXS with and without severe autistic behavior (FXS + ASD vs. FXS-only), who were compared to typically developing boys. In addition, we included levels of salivary cortisol before and after the assessment as a potential biomarker for ASD-related behaviors. Identifying risk factors for autism in FXS is critical to refine the phenotype, contribute to improved diagnostic specificity of autism in FXS, and identify potential preventive or treatment options.

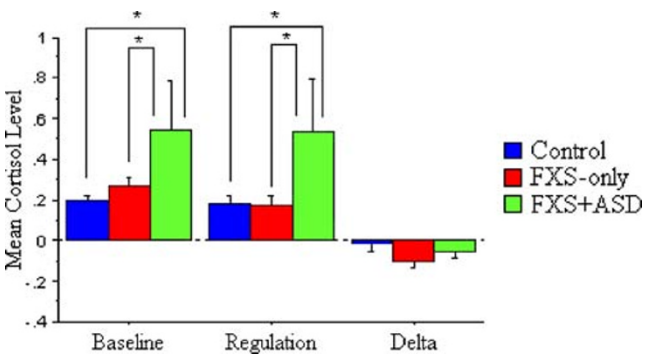

Fig. 2 Cortisol profiles in FXS. As depicted in these log transformed graph bars, the FXS + ASD group had significantly higher baseline and regulation cortisol levels than the FXS-only and control groups (*p<0.05). Although the FXS-only group had higher delta values, these differences were not significant

Our study indicates distinct social approach profiles on the SAS that uniquely differentiated typically developing boys from the FXS-only and FXS + ASD groups, and uniquely differentiated ASD behaviors across the two FXS groups. All three groups displayed the same trend of increased social approach with increased familiarity over time across the physical movement, facial expression, and eye contact scales. However, both groups of boys with FXS demonstrated elevated levels of social avoidance for facial expression and eye contact across initial and familiar encounters compared to the typically developing group. Avoidant physical movement appeared more complex and actually differentiated among the three groups over time. Specifically, both groups of boys with FXS were more avoidant than typically developing boys during initial encounters; however, only boys with FXS + ASD continued to be physically avoidant during familiar encounters, which differentiated them from both the typically developing group and FXS-only. Furthermore, correlations between CARS total scores as a continuum versus SAS measures in the entire FXS group revealed that autistic behavior was significantly related to a persistence of avoidant approach behavior. Consistent with our previous work [24], these findings indicate that the SAS is sensitive to identify both the pervasive social abnormalities that distinguish boys with FXS from typically developing peers, in general, and to differentiate boys with FXS with and without ASD. This

Table 3 Cortisol levels vs. measures of the SAS and CARS total score

		Baseline Cortisol	Regulation Cortisol	Delta Cortisol
Initial Approach	Physical Movement	–	0.0284[b]	
	Facial Expression	–	0.0009[a]	0.0092[a]
	Eye Contact	–	0.0155[a]	0.0456[a]
Familiar Approach	Physical Movement	–	–	–
	Facial Expression	0.0032[a]	–	–
	Eye Contact	0.0032[a]	–	–
	CARS Total Score	–	–	0.0077[b]

[a] Significant direct relationship in typically developing group

[b] Significant inverse relationship in FXS + ASD group

information refines the FXS phenotype by identifying specific time-sensitive dynamic social behaviors to consider when making differential diagnoses of autism in FXS and when developing treatments. The recognition that persistent social avoidance can differentiate children with FXS with autism from those who exhibit only initial social avoidance and do not have autism suggests that clinicians need to gather this information and treatment plans should be targeted to the pattern of social avoidance displayed by the child.

Consistent with the SAS profiles, cortisol analyses indicate a unique neuroendocrine profile for the boys with FXS + ASD. Boys with FXS + ASD had elevated baseline and regulation levels compared to both typicals and boys with FXS who were not different from each other. In contrast, delta (change) cortisol levels were not different among any of the groups. These findings are consistent with previous reports of elevated pre-assessment cortisol [27, 31] and similar cortisol change scores [27] in boys with FXS compared to typical siblings. However, our findings are unique in that we report cortisol abnormalities specific only to the FXS + ASD group whereas existing work does not differentiate between groups with FXS alone and those with FXS + ASD.

Integration of cortisol and behavior indicates that typically developing boys show strong relationships between cortisol and social approach on the SAS, with initial approach strongly correlated with cortisol reactivity and familiar approach with baseline cortisol levels, profiles that are consistent with existing literature on the general population [44–46]. In contrast, cortisol and SAS measures are weakly associated in the FXS + ASD group, only in terms of initial approach, and no relationship is found in the FXS-only group. Consistent with previous work [27], increased autistic behavior on the CARS is inversely correlated with change in cortisol (i.e., delta cortisol) in the group with FXS + ASD that contrasted with no relationship in the FXS-only group. The blunted cortisol regulation in boys with FXS + ASD is most likely related to their elevated baseline levels, which limits the range of cortisol reactivity. Nonetheless, it is interesting to note that the relationship with CARS scores was limited to the delta/change score and not to the elevated baseline or regulation levels given that group differences existed for the baseline and regulation scores and not the delta/change scores. Considering that the FXS + ASD group has already relatively elevated CARS scores, this intriguing result also suggests that there is a behavioral threshold for the relationship between autistic behavior and cortisol.

Our data showing that elevated cortisol and a relationship between CARS scores and cortisol was unique to the group with FXS + ASD, and not present in the FXS-only group, suggest that HPA dysfunction may be a biomarker for ASD in boys with FXS. HPA axis dysfunction as a biomarker for autism in FXS is partially supported by evidence from existing studies that indicate a relationship between elevated pre-assessment cortisol and increased autistic behaviors including gaze avoidance and poor eye contact in boys with FXS [19, 27]. However, these studies did not differentiate autism status within the group of boys with FXS so it is not clear if the relationship between cortisol and autism is pervasive across all boys with FXS or unique to those with elevated autistic behavior as we found in our study. Also, our study included a younger sample than reported in existing work (mean age 7.5 years compared to 10.8, 13.1, and 13.5; 19, 27, 30, 31), which extends the application of this biomarker to children in early to middle childhood. Given the strong evidence of cortisol stability across childhood [47–51], we do not believe our findings reflect blunted cortisol responses in young children, or in response to chronic stress, but further demonstrate that cortisol is a marker of autism in FXS. In contrast to our findings and existing work, a recent study reported a relationship between elevated pre-assessment

cortisol and lowered autistic behavior on the ADOS-G in FXS [19]. Nevertheless, this study did not examine regulation levels or changes in cortisol in relation to autistic behavior and defined the pre-assessment phase as the interval preceding the administration of the ADOS-G, which occurred mid-day after approximately 5 h of assessment and interaction [19], making direct comparisons across studies difficult.

Taken together, our work and others indicate a relationship between HPA function and autistic behavior in boys with FXS that is complex and interactive with environmental factors, including time spent in social interaction and the format and intensity of stressful events. Given that HPA dysfunction has also been implicated in idiopathic autism as reflected in abnormal baseline levels and circadian profiles [52–54] and exaggerated cortisol reactivity to social stresses in young children with ASD [53, 55–57], future studies including samples of children with FXS with and without ASD, as well as with idiopathic autism, would be highly informative about HPA axis function and its relationship with autistic behavior. The latter research should not only address the seemingly contradictory idiopathic ASD [52, 53, 58] and FXS literature [19, 27, 31] on cortisol levels and regulation, but also to advance our understanding of the causal relationship between cortisol (i.e., correlate or modifier) and autistic behavior.

The present study should be considered as a preliminary examination of the relationship between environmental and neuroendocrine factors and autistic behavior in FXS. Although our design included several strengths, such as the dynamic assessment of social approach behaviors by the SAS and cortisol measurements in the context of the SAS, there were also some shortcomings. The latter included the use of CARS and not the most contemporary ADI-R and ADOS-G for evaluating autistic behaviors, absence of measures of social anxiety, and variable and limited sample sizes for the different analyses. Additional cortisol measurements throughout a series of social interactions would have also been informative about the dynamics of cortisol regulation, as well as other indices of the HPA axis (e.g., ACTH). Certainly prospective, longitudinal studies that measure multiple factors including behavior, learning, gene and brain function, and environmental stressors in samples of individuals with FXS, typically developing children and relevant clinical groups such as idiopathic autism would be necessary to further delineate the role of environmental and neuroendocrine factors in autistic behavior in FXS.

Acknowledgments This research was supported by grants HD003110, HD40602, and MH067092. We thank Dr. Richard Thompson for his advice on statistical analyses.

References

1. Sherman SL, Marsteller F, Abramowitz AJ, Scott E, Leslie M, Bregman J. Cognitive and behavioral performance among FMR1 high-repeat allele carriers surveyed from special education classes. Am J Med Genet. 2002;114:458–65.
2. Kaufmann WE, Reiss AL. Molecular and cellular genetics of fragile X syndrome. Am J Med Genet. 1999;88:11–24.
3. Kaufmann WE, Cohen S, Sun HT, Ho G. Molecular phenotype of Fragile X syndrome: FMRP, FXRPs, and protein targets. Microsc Res Tech. 2002;57:135–44.
4. Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci. 2005;6:376–87.
5. Hagerman RJ, Hull CE, Safanda JF, Carpenter I, Staley LW, O'Connor RA, et al. High functioning fragile X males: demonstration of an unmethylated fully expanded FMR-1 mutation associated with protein expression. Am J Med Genet. 1994;51:298–308.
6. Kaufmann WE, Abrams MT, Chen W, Reiss AL. Genotype, molecular phenotype, and cognitive phenotype: correlations in fragile X syndrome. Am J Med Genet. 1999;83:286–95.
7. Loesch DZ, Bui QM, Huggins RM, Mitchell RJ, Hagerman RJ, Tassone F. Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. J Med Genet. 2007;44:200–4.
8. Bailey DB Jr, Hatton DD, Skinner M, Mesibov G. Autistic behavior, FMR1 protein, and developmental trajectories in young males with fragile X syndrome. J Autism Dev Disord. 2001;31:165–74.
9. Harris SW, Hessl D, Goodlin-Jones B, Ferranti J, Bacalman S, Barbato I, et al. Autism profiles of males with Fragile X syndrome. Am J Ment Retard. 2008;113:427–38.
10. Bennetto L, Pennington BF. Neuropsychology. In: Hagerman RJ, Hagerman PJ, editors. Fragile X syndrome: diagnosis, treatment, and research. 3rd ed. Baltimore: Johns Hopkins University Press; 2002. p. 206–48.
11. Hagerman RJ. The physical and behavioral phenotype. In: Hagerman RJ, Hagerman PJ, editors. Fragile X syndrome: diagnosis, treatment, and research. 3rd ed. Baltimore: Johns Hopkins University Press; 2002. p. 206–48.
12. Kau AS, Tierney E, Bukelis I, Stump MH, Kates WR, Trescher WH, et al. Social behavior profile in young males with fragile X syndrome: characteristics and specificity. Am J Med Genet A. 2004;126A:9–17.
13. Kaufmann WE, Cortell R, Kau AS, Bukelis I, Tierney E, Gray RM, et al. Autism spectrum disorder in fragile X syndrome: communication, social interaction, and specific behaviors. Am J Med Genet. 2004;129:225–34.
14. Budimirovic DB, Bukelis I, Cox C, Gray RM, Tierney E, Kaufmann WE. Autism spectrum disorder in Fragile X syndrome: differential contribution of adaptive socialization and social withdrawal. Am J Med Genet A. 2006;140A:1814–26.
15. Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L, et al. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. J Autism Dev Disord. 1989;19:185–212.
16. Lord C, Rutter M, Le Couteur A. Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord. 1994;24:659–85.
17. Philofsky A, Hepburn SL, Hayes A, Hagerman R, Rogers SJ. Linguistic and cognitive functioning and autism symptoms in young children with fragile X syndrome. Am J Ment Retard. 2004;109:208–18.
18. Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females

with fragile X full mutation and premutation. J Autism Dev Disord. 2007;37:738–47.
19. Hall SS, Lightbody AA, Reiss AL. Compulsive, self-injurious, and autistic behavior in children and adolescents with fragile X syndrome. Am J Ment Retard. 2008;113:44–53.
20. Cohen IL. Behavioral profiles of autistic and nonautistic fragile X males. Dev Brain Dys. 1995;8:252–69.
21. Bailey DB Jr, Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew LJ. Autistic behavior in young boys with fragile X syndrome. Autism Dev Disord. 1998;28:499–508.
22. Rogers SJ, Wehner DE, Hagerman R. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. J Dev Behav Pediatr. 2001;22:409–17.
23. Hatton DD, Hooper SR, Bailey DB, Skinner ML, Sullivan KM, Wheeler A. Problem behavior in boys with fragile X syndrome. Am J Med Genet. 2002;108:105–16.
24. Roberts JE, Weisenfeld LA, Hatton DD, Heath M, Kaufmann WE. Social approach and autistic behavior in children with fragile X syndrome. J Autism Dev Disord. 2007;37:1748–60.
25. Bailey DB Jr, Hatton DD, Mesibov G, Ament N, Skinner M. Early development, temperament, and functional impairment in autism and fragile X syndrome. J Autism Dev Disord. 2000;30:49–59.
26. Kaufmann WE, Capone GT, Clarke M, Budimirovic DB. Autism in genetic intellectual disability: insights into idiopathic autism. In: Zimmerman AW, editor. Autism: current theories and evidence. Totowa: The Humana Press Inc; 2008. p. 81–108.
27. Hessl D, Glaser B, Dyer-Friedman J, Reiss AL. Social behavior and cortisol reactivity in children with fragile X syndrome. J Child Psychol Psychiatry. 2006;47:602–10.
28. Hessl D, Rivera SM, Reiss AL. The neuroanatomy and neuroendocrinology of fragile X syndrome. Ment Retard Dev Disabil Res Rev. 2004;10:17–24.
29. Jacobson L. Hypothalamic-pituitary-adrenocortical axis regulation. Endocrinol Metab Clin North Am. 2005;34:271–92. vii.
30. Wisbeck JM, Huffman LC, Freund L, Gunnar MR, Davis EP, Reiss AL. Cortisol and social stressors in children with fragile X: a pilot study. J Dev Behav Pediatr. 2000;21:278–82.
31. Hessl D, Glaser B, Dyer-Friedman J, Blasey C, Hastie T, Gunnar M, et al. Cortisol and behavior in fragile X syndrome. Psychoneuroendocrinology. 2002;27:855–72.
32. Achenbach TM. Manual for the child behavior checklist/4–18 and 1991 Profile. Burlington: University Associates in Psychiatry; 1991.
33. Schopler E, Reichler RJ, Renner BR. The childhood autism rating scale (CARS). Los Angeles: Western Psychological Services; 1988.
34. Sturmey P, Matson JL, Sevin JA. Brief report: analysis of the internal consistency of three autism scales. J Autism Dev Disord. 1992;22:321–8.
35. Nordin V, Gillberg C, Nydén A. The Swedish version of the childhood autism rating scale in a clinical setting. J Autism Dev Disord. 1998;28:69–75.
36. Saemundsen E, Magnússon P, Smári J, Sigurdardóttir S. Autism diagnostic interview-revised and the childhood autism rating scale: convergence and discrepancy in diagnosing autism. J Autism Dev Disord. 2003;33:319–28.
37. Magyar CI, Pandolfi V. Factor structure evaluation of the childhood autism rating scale. J Autism Dev Disord. 2007;37:1787–94.
38. Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB Jr, Roberts J, et al. Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. Am J Med Genet A. 2006;140A:1804–13.
39. Goldsmith HH, Lemery KS. Linking temperamental fearfulness and anxiety symptoms: a behavior-genetic perspective. Biol Psychiatry. 2000;48:1199–209.
40. Magnano CL, Diamond EJ, Gardner JM. Use of salivary cortisol measurements in young infants: a note of caution. Child Dev. 1989;60:1099–101.
41. Schwartz EB, Granger DA, Susman EJ, Gunnar MR, Laird B. Assessing salivary cortisol in studies of child development. Child Dev. 1998;69:1503–13.
42. Sparrow SS, Balla DA, Cicchetti DV. Vineland adaptive behavior scales. Circle Pines: American Guidance Service; 1984.
43. Scheffe H. A method for judging all contrasts in the analysis of variance. Biometrika. 1953;40:87–104.
44. Smider NA, Essex MJ, Kalin NH, Buss KA, Klein MH, Davidson RJ, et al. Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: a prospective study. Child Dev. 2002;73:75–92.
45. Blair C, Peters R, Granger D. Physiological and neuropsychological correlates of approach/withdrawal tendencies in preschool: further examination of the behavioral inhibition system/behavioral activation system scales for young children. Dev Psychobiol. 2004;45:113–24.
46. Pérez-Edgar K, Schmidt LA, Henderson HA, Schulkin J, Fox NA. Salivary cortisol levels and infant temperament shape developmental trajectories in boys at risk for behavioral maladjustment. Psychoneuroendocrinology. 2008;33:916–25.
47. Bertrand PV, Rudd BT, Weller PH, Day AJ. Free cortisol and creatinine in urine of healthy children. Clin Chem. 1987;33:2047–51.
48. Gomez MT, Malozowski S, Winterer J, Vamvakopoulos NC, Chrousos GP. Urinary free cortisol values in normal children and adolescents. J Pediatr. 1991;118:256–8.
49. Phillip M, Aviram M, Leiberman E, Zadik Z, Giat Y, Levy J, et al. Integrated plasma cortisol concentration in children with asthma receiving long-term inhaled corticosteroids. Pediatr Pulmonol. 1992;12:84–9.
50. Kerrigan JR, Veldhuis JD, Leyo SA, Iranmanesh A, Rogol AD. Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. J Clin Endocrinol Metab. 1993;76:1505–10.
51. Knutsson U, Dahlgren J, Marcus C, Rosberg S, Brönnegård M, Stierna P, et al. Circadian cortisol rhythms in healthy boys and girls: relationship with age, growth, body composition, and pubertal development. J Clin Endocrinol Metab. 1997;82:536–40.
52. Curin JM, Terzić J, Petković ZB, Zekan L, Terzić IM, Susnjara IM. Lower cortisol and higher ACTH levels in individuals with autism. J Autism Dev Disord. 2003;33:443–8.
53. Corbett BA, Mendoza S, Wegelin JA, Carmean V, Levine S. Variable cortisol circadian rhythms in children with autism and anticipatory stress. J Psychiatry Neurosci. 2008;33:227–34.
54. Marinović-Curin J, Marinović-Terzić I, Bujas-Petković Z, Zekan L, Skrabić V, Dogas Z, et al. Slower cortisol response during ACTH stimulation test in autistic children. Eur Child Adolesc Psychiatry. 2008;17:39–43.
55. Corbett BA, Mendoza S, Abdullah M, Wegelin JA, Levine S. Cortisol circadian rhythms and response to stress in children with autism. Psychoneuroendocrinology. 2006;31:59–68.
56. Naber FB, Swinkels SH, Buitelaar JK, Bakermans-Kranenburg MJ, van IJzendoorn MH, Dietz C, et al. Attachment in toddlers with autism and other developmental disorders. J Autism Dev Disord. 2007;37:1123–38.
57. Lopata C, Volker MA, Putnam SK, Thomeer ML, Nida RE. Effect of social familiarity on salivary cortisol and self-reports of social anxiety and stress in children with high functioning autism spectrum disorders. J Autism Dev Disord. 2008;38:1866–77.
58. Tordjman S, Anderson GM, McBride PA, Hertzig ME, Snow ME, Hall LM, et al. Plasma beta-endorphin, adrenocorticotropin hormone, and cortisol in autism. J Child Psychol Psychiatry. 1997;38:705–15.