The American Journal of Psychiatry

Neurobiology of Self-Regulation: Longitudinal Influence of FKBP5×Intimate Partner Violence on Emotional and Cognitive Development in Childhood

Journal:	*The American Journal of Psychiatry*
Manuscript ID	AJP-18-09-1018.R1
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Halldorsdottir, Thorhildur; Max Planck Institute of Psychiatry, Dept. of Translational Research in Psychiatry; Haskoli Islands, Kurtoic, Dunja; Max Planck Institute of Psychiatry, Dept. of Translational Research in Psychiatry Mueller-Myhsok, Bertram; Max-Planck Institute of Psychiatry, Translational Research in Psychiatry ; Munich Cluster for Systems Neurology (SyNergy) Binder, Elisabeth; Max-Planck Institute of Psychiatry, Translational Research in Psychiatry ; Emory University, Psychiatry and Behavioral Sciences Blair, Clancy; Department of Human Development and Family Studies, Pennsylvania State University
Keywords:	Biological Markers - AJP0082, Child Psychiatry - AJP0102

Word count: 3,889

Number of tables and figures: 4

Neurobiology of Self-Regulation: Longitudinal Influence of *FKBP5* × Intimate Partner Violence on Emotional and Cognitive Development in Childhood

Thorhildur Halldorsdottir[1,2*], PhD, Dunja Kurtoic[1], MS, Prof. Bertram Mueller-Mysok[1,3], PhD, & The Family Life Project Key Investigators[6] Prof. Elisabeth B. Binder[1,4+], PhD, Prof. Clancy Blair[5*+], PhD,

[1] Department of Translational Research in Psychiatry, Max Planck Institute of Psychiatry

[2] Center of Public Health Sciences, University of Iceland

[3] Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool

[4] Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine

[5] Department of Applied Psychology, New York University

[6] Department of Human Development and Family Studies, Penn State University and Frank Porter Graham Child Development Institute, University of North Carolina Chapel Hill.

[+]These authors contributed equally to the project

[*] Corresponding authors:

Thorhildur Halldorsdottir,
Department of Translational Research in Psychiatry,
Kraepelinstrasse 2-10,
Munich, 80804, Germany
Telephone: +49 89 306221
Email: halldorsdottir@psych.mpg.de

Clancy Blair,
Department of Applied Psychology,
Steinhardt School of Culture Education and Human Development New York University
246 Greene St, Kimball Hall, 8th floor
New York NY 10003
Telephone: (212) 998-5853
Email: clancy.blair@nyu.edu

Keywords: Early life stress, self-regulation, *FKBP5*, gene-by-environment

ABSTRACT

Objective: Self-regulation includes the volitional and non-volitional regulation of emotional, cognitive, and physiological responses to stimulation. It develops from infancy through individual characteristics and the environment, with the stress hormone system as a central player. Accordingly, genes involved in regulating the stress system –such as FK506-binding protein 5 (*FKBP5*)- are hypothesized to interact with early life stress exposure, such as intimate partner violence (IPV), to predict self-regulation indicators and associated outcomes, such as behavioral and learning problems in school.

Methods: Participants comprised a longitudinal birth cohort of 910 children with *FKBP5* genotypes and assessed for exposure to IPV prior to age 2 and multiple measures of self-regulation: stress-induced cortisol reactivity and fear-elicited emotional reactivity at 7, 15, and 24 months, executive function (EF) at 36, 48, and 60 months, and emotional and behavioral difficulties and reading and math achievement in grades 1, 2, and 5. Data were analyzed using longitudinal clustering and ordinal logistic regression procedures followed by mixed linear modeling.

Results: Youth with two copies of a risk *FKBP5* haplotype and IPV exposure were significantly more likely to belong to a developmental trajectory characterized by high prolonged stress-induced cortisol reactivity and emotional reactivity in toddlerhood, followed by low EF at school entry, and high emotional and behavior problems and low reading ability in primary school grades.

Conclusions: *FKBP5*×IPV impacts the physiological response to stress early in life with consequences for emotional and cognitive self-regulation. Targeting self-regulation may present an early intervention strategy for children facing genetic and environmental risk.

PLAIN LANGUAGE SUMMARY

Self-regulation encompasses interconnected attributes associated with mental health and educational outcomes across the lifespan. Self-regulation develops through the interplay between individual characteristics and environmental influences via the stress hormone system. In this study, we examined whether early life stress in the form of intimate partner violence, a common form of domestic violence, interacts with *FKBP5* - a gene involved in stress responsivity - to predict stress-induced cortisol and emotional reactivity in infancy, followed by emotional, behavioral and cognitive outcomes associated with self-regulation across early development. Starting with heightened cortisol levels following a stressor and high emotional reactivity in toddlers, our findings suggest continuity in self-regulation difficulties extending to middle childhood based on *FKBP5* genotype and intimate partner violence exposure. These findings could inform early intervention strategies with consequences for outcomes later in development.

INTRODUCTION

Exposure to early life stress (ELS) robustly predicts a wide range of negative outcomes throughout the lifespan(1-3). Intimate partner violence (IPV) is a prevalent form of ELS that refers to a pattern of behavior in which one intimate partner threatens, intimidates, isolates, coerces, or uses emotional, sexual, or economic abuse to control the other partner(1). There is, however, marked variability in outcomes following ELS, raising questions about individual differences in genetic vulnerability to adverse environments. Identifying factors contributing to negative outcomes following ELS is of great clinical importance and may inform interventions to enhance resilience.

Several genetic polymorphisms in genes involved in the stress response, mainly in the hypothalamic-pituitary adrenal (HPA) axis, are thought to moderate the impact of adversity on negative outcomes(4). Among the genes more consistently found to moderate the relationship between early adversity and negative outcomes is FK506-binding protein 5 (*FKBP5*). *FKBP5* plays an important role in the HPA axis through regulating the glucocorticoid receptor (GR) complex. *FKBP5* provides an ultra-short feedback of GR-sensitivity by being transcriptionally activated by the GR and subsequently limiting GR activity via protein-protein interactions with systemic consequences on the HPA-axis' set point (for review, see (5)). This molecular feedback regulation is moderated by common genetic variations. Four single-nucleotide polymorphisms (SNPs; i.e., rs9296158, rs3800373, rs1360780, and rs9470080) within a functional haplotype in the *FKBP5* locus have received the most attention. Molecular studies have shown that the CATT haplotype leads to an enhanced induction of FKBP5 expression with exposure to glucocorticoids by altering the 3D structure of the locus(6-10). Increased FKBP5 mRNA expression in postmortem brains of patients with psychiatric disorders and animal studies using transgenic,

viral and pharmacological tools support that increased expression of FKBP5, especially in the hippocampus and the amygdala leads to increased anxiety and decreased stress coping (for recent review, see (5)). This may suggest that increased FKBP5 expression driven by genetic and environmental factors could contribute to psychiatric disorders. Indeed, this high expression haplotype, when combined with exposure to early adversity, predicts a host of psychiatric symptoms and traits, including depression, post-traumatic stress disorder, psychosis and suicide attempts, as well as impaired learning and memory in adulthood(5). Given these broad associations observed, one could hypothesize that these diverse outcomes in adulthood may be driven by similar mechanisms early in life, with a common risk trajectory in childhood, followed by divergent symptom presentations later in life. In support of this notion, effects of *FKBP5*×ELS on known early risk factors for the development of psychiatric symptoms have been documented in youth, ranging from greater stress-induced cortisol reactivity in infancy to heightened risk for anxiety, depression, substance use and dissociation during adolescence (see (5), for review).

Expanding upon previous findings, in this study we investigate interrelated aspects of self-regulation as a potential common pathway through which ELS×*FKBP5* genotype increases risk for diverse negative outcomes, including psychopathology risk and poor academic achievement, in a longitudinal dataset. We define self-regulation as a bidirectional system composed of cognitive, emotional, and physiological components that are hierarchically organized and reciprocally integrated. In its mature form, self-regulation is characterized by reflective, 'top-down' control of behavior, in which executive function (EF) and the volitional control of attention can regulate emotional and physiological arousal in ways that promote engagement in goal-directed actions. Self-regulation, however, is also characterized by reactive,

'bottom-up' control of behavior in which levels of physiological arousal associated with the stress response and emotional reactivity can support or undercut the brain's capacity for volitional 'top-down" control(11). Mechanistically, these associations are driven by hormones (glucocorticoids and monoamines) that at moderate levels increase neural activity in prefrontal cortex (PFC) but at high levels shutdown activity in PFC and increase activity in the amygdala. As such, the effective regulation of emotion and the physiological response to stress in the infant and toddler periods sets the stage for the development of higher-order self-regulation abilities, such as EF(12, 13). Early emotion regulation and EF abilities have been associated with a broad range of outcomes later in life, including mental health outcomes, school readiness, and academic achievement(12).

From infancy onward, self-regulation develops through the interplay between individual characteristics and environment via the stress hormone system, with consequences for brain development(13). Animal models have demonstrated that increased corticosteroid levels in response to ELS alter gene expression and induce structural and connectivity changes in brain areas involved in the regulation of stress response physiology(14-17), which modulates the neuroendocrine systems that underlie behavioral responses to stress. Variation in neural connectivity influences inter-individual variation in stress physiology(17). Through this interplay, ELS can have wide-ranging effects on aspects of social-emotional and cognitive development as the self-regulation system adapts to conditions of high threat and vigilance.

Building on this empirical and theoretical basis, we hypothesized that the combined effects of ELS and *FKBP5* are expected to influence stress-induced cortisol reactivity and phenotypes associated with impaired self-regulation. To test this hypothesis, this study investigates the interaction between IPV exposure over the child's first two years and functional

FKBP5 haplotypes in shaping self-regulation abilities across developmental stages. The self-regulation abilities examined in this study are prolonged stress-induced cortisol reactivity and fear-elicited emotional reactivity in the infant/toddler period, EF in early childhood, and emotional and behavioral difficulties and reading and math abilities across primary school grades (see Figure S1 for schematic figure).

METHODS

Participants

The analysis sample comprised 910 children (461 females, 50.7%; 518 African American, 56.9%; and 392 White, 43.1%) with genetic data from the Family Life Project (FLP), a longitudinal birth cohort of families living in predominantly low-income and rural communities in the U.S.(18). For a detailed description of the sample, sampling procedure and data collection, see the supplement and Vernon-Feagans & Cox (2013)(18).

Measures

Intimate Partner Violence (IPV). The child's primary caregiver, in almost all instances the child's biological mother (95%), reported on IPV using the Conflict Tactics Scale(19) at child ages 7, 15, and 24 months. An aggregate of the respondent's own and the respondent's partner's physical and psychological aggression was created to a quantitative index violence exposure (range 0-4) during the child's first two years.

Fear-Elicited Emotional Reactivity. At 7, 15, and 24 months children were administered a mask presentation procedure to induce fear(20, 21). The experimenter wore four different masks, one at a time, while moving slowly from side to side in front of the child, saying the child's name using a neutral tone of voice. At 7 months, children were also administered a barrier procedure and arm restraint procedure, and at 15 and 24 months children were administered a toy removal procedure to induce frustration. We focus on the mask procedure given that it has been associated with the fear response and activity in corticolimbic neural circuitry(22) that is central to our theoretical model. See supplemental for details on coding, psychometric properties and

Figure S2 for cortisol levels prior to and following the stress paradigm at each time point broken down by CATT haplotype and IPV exposure.

Prolonged Stress-Induced Cortisol Reactivity. Stress-induced changes in cortisol were measured at 7, 15, and 24 months. To assess stress-induced changes in cortisol, saliva samples were collected prior to the mask presentation and at 20 and 40 minutes post peak arousal to the procedure and later assayed for cortisol. Further description of the measurement of cortisol and definition of peak arousal is described in the supplement.

Intellectual Ability. The Mental Development Index (MDI) of the Bayley Scales of Infant Development(23), administered at 15 months, and the full scale estimate of IQ from the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III)(24), administered at 36 months, were used to assess the child's cognitive abilities.

Maternal Depressive Symptoms. Self-reported maternal depressive symptoms at child age 24 months were assessed with the Center for Epidemiological Studies–Depression Scale (CES-D)(25).

Executive Function. At 36, 48, and 60 months, children were administered an EF assessment battery that included three inhibitory control tasks (Spatial Conflict Arrows, Silly Sounds Stroop, Animal Go No-Go), two working memory tasks (Working Memory Span, Pick the Picture), and one attention-shifting task (Something's the Same)(26). See supplement and Willoughby and colleagues (2012)(26) for a detailed description of the tasks and the administration procedures.

Emotional and Behavioral Problems. The child's lead teacher in school at grades 1, 2, and 5 completed the Strengths and Difficulties Questionnaire (SDQ)(27) to assess conduct problems, hyperactivity, emotional problems, and peer problems. A total score of the SDQ subscales was used to measure emotional and behavioral problems over time.

Reading and Math Ability. In grades 1, 2, and 5, the Letter-Word Identification and Applied Problems subtests of the Woodcock-Johnson III Tests of Achievement(28) were administered to assess reading and math ability. W scores, appropriate for longitudinal analysis of change, were used in all analyses.

Genotypes. DNA was extracted from both the child and the biological mother and genotyping of rs9296158, rs3800373, rs1360780, and rs9470080 was conducted with the appropriate probes for a Taqman SNP Genotyping Assay using an Allelic Discrimination Assay protocol (Applied Biosystems, Foster City, CA). In addition, genetic ancestry markers were assessed. See the supplemental for genotyping and quality control procedures and genetic ancestry marker analyses. These four *FKBP5* SNPs were combined to determine the copy numbers of the rarer CATT haplotype carried by each individual using PLINK (children: 432 non-carriers, 384 one CATT haplotype allele carriers, and 94 two CATT haplotype allele carriers; mothers: 414 non-carriers, 365 one CATT haplotype allele carriers and 86 two CATT haplotype allele carriers). Haploview was used to determine the LD structure of the *FKBP5* SNPs (Figure S3).

Covariates. Covariates included child sex, child state of residence, cumulative risk, household chaos, and genetic ancestry markers. Cumulative risk is a continuous score based on family income-to-needs ratio, constant spouse/partner living in the home, maternal education, hours of employment, household density, occupational prestige, and neighborhood noise/safety measured at 7, 15, and 24 months postpartum(18). Household chaos is a continuous score based on the number of times the child moved to residence, changes in the primary and secondary caregiver, changes in the people within the household at 7, 15, and 24 months. These scores are based on previous work with the FLP(18). To control for population stratification, the first 6 principal

components derived from the ancestry specific genotypes (see supplemental methods) were included in all analyses.

Statistical Analyses

Genotype differences in demographic and clinical characteristics were examined with chi-square and regression models. To examine prolonged stress-induced cortisol reactivity, we regressed cortisol levels from the earlier time points (cortisol levels at baseline and 20 minutes after the stress paradigm) and the covariates on to the cortisol levels 40 minutes following the stressor at each assessment (7, 15, and 24 months). The residuals from these models were used in our longitudinal cluster analyses. Multiple imputation was used to handle missing data (see supplemental for imputation procedures and Table S1 for available data by outcome).

Analyses followed an additive genetic model in which individuals with increasing numbers of risk haplotypes (i.e., non-carrier=0, one CATT haplotype allele=1, and 2 CATT haplotype alleles=2) were compared and exposure to IPV was examined as a continuous measure. K-means based longitudinal clustering was conducted to determine groups of participants with similar phenotype presentation across time. Two separate cluster analyses were conducted to account for the developmental stage of measurement. The first cluster analysis included prolonged stress-induced cortisol reactivity and fear-elicited emotional reactivity measured at 7, 15, and 24 months. The second cluster analysis included reading and math ability and teacher-reported emotion and behavioral difficulties at grades 1, 2, and 5. The best clustering solution was determined using the Calinski & Harabatz criterion (CH index)(29). To assess developmental progression of youth from the early development clusters to the school-

aged clusters, Cohen's Kappa test(30) was run to infer the correspondence between two clustering results. See supplemental for details on the cluster analyses procedures.

The outcomes of the early development and school-aged cluster analyses were ranked based on phenotype severity. Ordinal logistic regression(31) with a proportional odds regression was used to examine the main effect model (G or E), additive model (G + E), and multiplicative interactive model (G×E) of the CATT haplotype and IPV exposure on the ranked phenotypes. To determine model fit, likelihood ratio tests were used to compare each model to the reduced model. To complement these analyses, mixed linear growth models were conducted in R to illustrate the findings of the longitudinal cluster analyses for each outcome (see supplemental).

We carried out several sensitivity analyses to exclude alternative explanations and determine the specificity of the effects. These analyses are described in the supplementary materials and Figure S4.

RESULTS

No differences in demographics or clinical characteristics were noted based on child genotype (p >0.05) (see Table S2 in supplemental). For child outcomes, the correlation was lower among variables earlier in development compared to outcomes in early and middle childhood (see Figure S5). However, the correlation between outcomes gradually increased across variables from 24 months onward, suggesting that convergences of developmental trajectories over the first 24 months are relevant for the prediction of later outcome.

Longitudinal Cluster Analyses

Longitudinal cluster analyses were used to identify individuals with similar prolonged stress-induced cortisol reactivity and emotion regulation over the first 2 years of life and behavioral and academic trajectories from grade 1 to 5. For both the early development and school-aged cluster analyses, a two-cluster solution had the highest CH index (Figure S6). During early development, a cluster with low emotional reactivity and faster normalization of cortisol (Cluster low emotional reactivity/cortisol; 51.1% of participants; Figure S7) separated from a cluster of children with higher emotional reactivity and prolonged normalization of cortisol (Cluster high emotional reactivity/cortisol, 48.9% of participants). In the school-aged children, a cluster with less behavioral problems and higher academic ability (Cluster low behavioral problems/high academic achievement; 63.4% of participants) contrasted with a cluster with more behavioral problems and lower academic ability (Cluster high behavioral problems/low academic achievement; 36.6% of participants). The clusters across the two developmental stages were in 'slight agreement' (Cohen's Kappa=0.032[95% CI -0.034-0.097], p=0.17).

The combined developmental clusters were ranked based on severity of difficulties across time. Figure 1 illustrates all the outcomes according to the four cluster groups. Youth within the clusters of high emotional reactivity/cortisol in toddlerhood and high behavioral problems/low academic achievement later on had the most difficulties across time (High stress sensitivity toddler/high risk school-aged, n=170). They were followed by children who presented with high levels of difficulties in the school-aged analyses but not increased emotional reactivity during early development (Low stress sensitivity toddler/high risk school-aged, n=163). A third group of youth presented with increased emotional reactivity difficulties during early childhood but no difficulties in middle childhood (High stress sensitivity toddler/low risk school-aged, n=275). Youth in the last group presented with both low emotional reactivity during early childhood and low emotion and behavioral difficulties and high reading ability in middle childhood (Low stress sensitivity toddler/low risk school-aged, n=302).

Ordinal logistic regression analysis

Ordinal logistic regression revealed that EF over development, which was not used to define the cluster groups and measured at timepoints between the early- and school-aged trajectories (36, 48 and 60 months), was significantly associated with the cluster groups (b=0.246, se=0.034, $p<0.001$, Figure 1C). Youth with elevated levels of prolonged stress-induced cortisol and emotional reactivity in toddlerhood also had the lowest EF at all three timepoints.

Next, we examined how the IPV exposure and *FKBP5* haplotypes interaction was associated with the developmental trajectory a child belonged to. A model including the interaction of CATT haplotype×IPV exposure showed a stronger association with the cluster groups than both the null model ($p=0.005$) and the additive model ($p=0.037$; see Table 1 for

model comparisons), providing evidence that the CATT haplotype×IPV interaction is associated with the clustering orders (Figure 2). Youth belonging to the high stress sensitivity toddler/high risk school-aged cluster had the highest IPV exposure and the highest proportion of the two copies of the CATT risk haplotype carriers. Specifically, 29.7% of participants carrying two copies of the CATT haplotype and exposed to above average levels of IPV exposure (IPV_{mean}=0.859) belonged to the high stress sensitivity toddler/high risk school-aged cluster compared to 14.2% of participants carrying two copies of the CATT haplotype with lower levels of IPV exposure. The odds ratio of carrying two copies of the CATT haplotype and having high IPV exposure and being in the group of children with an at-risk developmental trajectory (i.e., high stress sensitivity toddler/high risk school-aged cluster) is 4.15 (p= 0.031, 95% CI 0.99–21.12) in comparison to being in the group of children with a low risk developmental trajectory (i.e., low stress sensitive toddler/low risk school-aged cluster).

Mixed linear models

Mixed models analyses were used to more explicitly visualize the findings from the longitudinal cluster analyses. The CATT haplotype×IPV interaction significantly predicted prolonged stress-induced cortisol and emotional reactivity at 15 and 24 months (p=0.035 and p=0.038, respectively), EF at 60 months (p=0.029), emotional and behavioral problems (p=0.020) and reading ability (p=0.035) at grades 1, 2, and 5 (Cohen's D ranging from 0.13 for prolonged stress-induced cortisol at 15 months to 0.77 for emotional and behavioral problems in grade 5 for the significant findings). These analyses are visualized in Figure 3 and detailed further in the supplemental results section.

Sensitivity Analyses

The IPV×CATT haplotype remained a significant predictor of reading and math ability after adjusting for general intelligence. The interaction also remained significant predictor of cluster group assignment when controlling for maternal depressive symptoms. Maternal CATT haplotype, independently or in combination with IPV, was not associated with cluster group assignment. The interaction between cumulative risk and CATT haplotype did not significantly predict any of the self-regulation or school age outcomes (p>0.050). The IPV×CATT haplotype×ethnicity predicting cluster groups was nonsignificant (p=0.250). Lastly, the CATT haplotype×IPV did not predict any measure of general intelligence, providing support for the interaction effect being specific to self-regulation outcomes.

DISCUSSION

As predicted by the model of self-regulation, exposure to high levels of IPV prior to age 2 among *FKBP5* risk haplotype carriers was associated with higher levels of physiological and emotional reactivity with consequences for emotional and cognitive development through middle childhood.

The findings are consistent with the mounting research on the dynamic relationship between the stress hormone system and self-regulation and how their interaction affects an individual's functioning over time(32). The associations were confined to self-regulation outcomes and not observed for intellectual ability. Previous molecular and endocrine findings show that an enhanced up-regulation of *FKBP5* during the acute stress phase (which has been associated with the CATT haplotype) leads to impaired negative feedback of the stress hormone system and prolonged cortisol responses by reducing GR sensitivity and thus negative feedback mechanisms (for review, see (5)). Therefore, an altered endocrine response to stress in the presence of a chronic stressor, such as IPV, would be expected to influence behavioral outcomes. In accordance, the collective findings of this study indicate that children carrying the CATT haplotype and exposed to IPV during the first two year of life are significantly more likely to display early prolonged cortisol responses in parallel with differences in emotion regulation, followed by later cognitive and behavioral differences. This may suggest that early variation in emotion- and stress hormone response regulation determined by environmental and genetic risk may drive aspects of behavioral adaptation over development.

The observed behavioral differences in this study may be the result of increased FKBP5 expression in the developing brain. In experimental animals, higher FKBP5 expression in specific brain regions has been associated with increased anxiety related behavior, as well as

reduced stress coping (for review, see (35)). Human neuroimaging studies have shown an association between the high *FKBP5* expression CATT haplotype and changes in the volume and connectivity of the hippocampus, frontal cortex and amygdala(33). Furthermore, a recent postmortem study revealed decreased synaptic spine density in the medial orbitofrontal cortex associated with increased FKBP5 expression (which would be predicted in CATT carriers with ELS exposure(34)), suggesting high FKBP5 expression may be associated with the biological systems necessary for learning and memory(35). In fact, increases in FKBP5 would not only impact GR-regulation, but alter a number of downstream pathways that FKBP5 influences via protein/protein interaction(36). These include systems associated with neuronal function and synaptic plasticity and could thus directly impact these brain circuits. Future studies are needed to examine *FKBP5*×ELS, such as IPV exposure, effects on emotional circuit activation throughout development.

The current study adds to the growing literature indicating that *FKBP5* and ELS interact in shaping outcomes across multiple domains of functioning(5). In this study, the clusters at each developmental stage were in 'slight agreement' according to the Cohen's Kappa. This relatively low correlation between child outcomes and the clusters likely reflects processes of equifinality (multiple pathways ending with the same outcome) and multifinality (children with similar risks ending with different outcomes)(37, 38), as is commonly presented in the literature(37). Moreover, our analyses were confined to exposure to IPV prior to age 2 and we did not adjust for environmental influences after that period. Importantly, despite not controlling for environmental influences past the age of 2 years old, we still find that children characterized by the combination of the *FKBP5* risk haplotype and IPV exposure prior to age 2 are overrepresented in the high-risk outcome trajectories across all age periods.

This study has a number of strengths (e.g., multi-informant approach, biological measures, longitudinal assessment), but also several limitations. First, participants with missing IPV data were more likely to be African American, and to be characterized by higher cumulative risk. In an effort to overcome this limitation, we employed state-of-the-art procedures to address missing data (see supplement). Second, the diverse ethnic background of the cohort may confound the G×E analysis. To avoid false positive results confounded by population stratification, we used ancestry informative genetic markers as covariates in all analyses and tested for the three-way interaction of IPV with *FKBP5* and race/ethnicity. We found no evidence that ethnicity confounds the observed G×E. Similarly, our sample was composed of predominantly low socioeconomic status youth. Poverty is a well-studied ELS associated with poor physical and mental health. Importantly, cumulative risk, which incorporated SES, did not significantly predict prolonged stress-induced cortisol reactivity and emotional reactivity in this study, whereas exposure to IPV did. Hence, in order to investigate how factors that influence prolonged stress-induced cortisol reactivity and emotional reactivity in early childhood impact later outcomes, we believe that IPV is the best suited exposure in this cohort. Third, it was beyond the scope of the study to disentangle whether the increased risk for behavioral symptoms and poor academic achievement observed were due to other stressors, e.g., prenatal stress due to IPV exposure during gestation. The association of other types of early adversity with HPA reactivity and longitudinal child outcomes remain an important future research venture. Fourth, the present study focused on G×E influencing self-regulation from infancy to middle childhood. A recent cross-sectional study suggests that *FKBP5* may interact with ELS to predict self-regulation outcomes, namely rumination and catastrophizing, during adolescence(39). Further

longitudinal data are required to determine whether this interaction influences adult self-regulation outcomes.

In conclusion and consistent with the hypothesized model of self-regulation, functional *FKBP5* haplotypes interacted with IPV exposure resulting in long-term and likely interconnected effects on endocrine, emotional and cognitive functioning throughout development. These findings provide valuable insight into potential early shared mechanisms underlying the diverse mental health and cognitive outcomes associated with early adversity×*FKBP5* interactions and could have important implications in terms of identifying at-risk youth and intervention targets.

ACKNOWLEDGMENTS

We would like to thank the many families and research assistants who made this study possible. Support for this research was provided by the National Institute of Child Health and Human Development grants R01 HD51502 and P01 HD39667 with co-funding from the National Institute on Drug Abuse and by the National Institutes of Health Environmental Influences on Child Health Outcomes program grant UG3OD023332.

TH was in part funded by ERC Consolidator Grant (StressGene, grant no:726413, Dr Valdimarsdóttir).

The Family Life Project Key investigators include: Lynne Vernon-Feagans, The University of North Carolina at Chapel Hill; Mark T. Greenberg, The Pennsylvania State University; Clancy B. Blair, New York University; Margaret R. Burchinal, The University of North Carolina at Chapel Hill; Martha Cox, The University of North Carolina at Chapel Hill; Patricia T. Garrett-Peters, The University of North Carolina at Chapel Hill; Jennifer L. Frank, The Pennsylvania State University; W. Roger Mills-Koonce, University of North Carolina-Greensboro; and Michael T. Willoughby, RTI International.

REFERENCES

1. Vu NL, Jouriles EN, McDonald R, Rosenfield D. Children's exposure to intimate partner violence: a meta-analysis of longitudinal associations with child adjustment problems. Clinical psychology review. 2016;46:25-33.
2. Koenen KC, Moffitt TE, Caspi A, Taylor A, Purcell S. Domestic violence is associated with environmental suppression of IQ in young children. Development and psychopathology. 2003;15:297-311.
3. Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Aguilar-Gaxiola S, Alhamzawi AO, Alonso J, Angermeyer M. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. The British Journal of Psychiatry. 2010;197:378-385.
4. Halldorsdottir T, Binder EB. Gene x Environment Interactions: From Molecular Mechanisms to Behavior. Annual review of psychology. 2017;68:215-241.
5. Matosin N, Halldorsdottir T, Binder EB. Understanding the Molecular Mechanisms Underpinning Gene by Environment Interactions in Psychiatric Disorders: The FKBP5 Model. Biological psychiatry. 2018;83:821-830.
6. Luijk MP, Velders FP, Tharner A, van Ijzendoorn MH, Bakermans-Kranenburg MJ, Jaddoe VW, Hofman A, Verhulst FC, Tiemeier H. FKBP5 and resistant attachment predict cortisol reactivity in infants: gene-environment interaction. Psychoneuroendocrinology. 2010;35:1454-1461.
7. Buchmann AF, Holz N, Boecker R, Blomeyer D, Rietschel M, Witt SH, Schmidt MH, Esser G, Banaschewski T, Brandeis D. Moderating role of FKBP5 genotype in the impact of childhood adversity on cortisol stress response during adulthood. European Neuropsychopharmacology. 2014;24:837-845.
8. Ising M, Depping AM, Siebertz A, Lucae S, Unschuld PG, Kloiber S, Horstmann S, Uhr M, Muller-Myhsok B, Holsboer F. Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. The European journal of neuroscience. 2008;28:389-398.
9. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. Jama. 2008;299:1291-1305.
10. Touma C, Gassen NC, Herrmann L, Cheung-Flynn J, Bull DR, Ionescu IA, Heinzmann JM, Knapman A, Siebertz A, Depping AM, Hartmann J, Hausch F, Schmidt MV, Holsboer F, Ising M, Cox MB, Schmidt U, Rein T. FK506 binding protein 5 shapes stress responsiveness: modulation of neuroendocrine reactivity and coping behavior. Biological psychiatry. 2011;70:928-936.
11. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. Nature Reviews Neuroscience. 2009;10:410.
12. Blair C, Raver CC. School readiness and self-regulation: a developmental psychobiological approach. Annual review of psychology. 2015;66:711-731.
13. Blair C, Raver CC. Child development in the context of adversity: experiential canalization of brain and behavior. The American psychologist. 2012;67:309-318.
14. Teicher MH, Samson JA. Annual research review: enduring neurobiological effects of childhood abuse and neglect. Journal of child psychology and psychiatry. 2016;57:241-266.

15. Teicher MH, Samson JA. Childhood maltreatment and psychopathology: A case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. American journal of psychiatry. 2013;170:1114-1133.
16. Teicher MH, Samson JA, Anderson CM, Ohashi K. The effects of childhood maltreatment on brain structure, function and connectivity. Nature Reviews Neuroscience. 2016;17:652.
17. Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ. Neurobiology of resilience. Nature neuroscience. 2012;15:1475-1484.
18. Vernon-Feagans L, Cox M. The Family Life Project: an epidemiological and developmental study of young children living in poor rural communities. Monographs of the Society for Research in Child Development. 2013;78:1-150.
19. Straus MA, Hamby SL, Boney-McCoy S, Sugarman DB. The revised conflict tactics scales (CTS2) development and preliminary psychometric data. Journal of family issues. 1996;17:283-316.
20. Ursache A, Blair C, Stifter C, Voegtline K. Emotional reactivity and regulation in infancy interact to predict executive functioning in early childhood. Developmental psychology. 2013;49:127-137.
21. Goldsmith H, Rothbart MK. Prelocomotor and locomotor laboratory temperament assessment battery (Lab-TAB; version 3.0, technical manual). Madison: University of Wisconsin, Department of Psychology. 1996.
22. LeDoux J. Rethinking the emotional brain. Neuron. 2012;73:653-676.
23. Bayley N: Bayley scales of infant development: Manual, Psychological Corporation; 1993.
24. Wechsler D: WPPSI-III administration and scoring manual, Psychological Corporation; 2002.
25. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Applied psychological measurement. 1977;1:385-401.
26. Willoughby MT, Blair CB, Wirth RJ, Greenberg M. The measurement of executive function at age 5: psychometric properties and relationship to academic achievement. Psychological assessment. 2012;24:226.
27. Goodman R. The Strengths and Difficulties Questionnaire: a research note. Journal of child psychology and psychiatry. 1997;38:581-586.
28. Woodcock RW, McGrew KS, Mather N: Woodcock-Johnson III tests of cognitive abilities, Riverside Pub.; 2001.
29. Caliński T, Harabasz J. A dendrite method for cluster analysis. Communications in Statistics-theory and Methods. 1974;3:1-27.
30. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159-174.
31. Agresti A: Categorical Data Analysis. 2nd edition ed. New Jersey: Hoboken, John Wiley & Sons, Inck; 2002.
32. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature reviews Neuroscience. 2009;10:434-445.
33. Tozzi L, Carballedo A, Wetterling F, McCarthy H, O'Keane V, Gill M, Morris D, Fahey C, Meaney J, Frodl T. Single-Nucleotide Polymorphism of the FKBP5 Gene and Childhood Maltreatment as Predictors of Structural Changes in Brain Areas Involved in Emotional Processing in Depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2016;41:487-497.

34. Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nature neuroscience. 2013;16:33-41.
35. Young KA, Thompson PM, Cruz DA, Williamson DE, Selemon LD. BA11 FKBP5 expression levels correlate with dendritic spine density in postmortem PTSD and controls. Neurobiology of stress. 2015;2:67-72.
36. Rein T. FK506 binding protein 51 integrates pathways of adaptation: FKBP51 shapes the reactivity to environmental change. BioEssays : news and reviews in molecular, cellular and developmental biology. 2016;38:894-902.
37. Cicchetti D. Annual research review: Resilient functioning in maltreated children–past, present, and future perspectives. Journal of child psychology and psychiatry. 2013;54:402-422.
38. Cicchetti D, Rogosch FA. Equifinality and multifinality in developmental psychopathology. 1996.
39. Halldorsdottir T, de Matos APS, Awaloff Y, Arnarson EÖ, Craighead WE, Binder EB. FKBP5 Moderation of the Relationship between Childhood Trauma and Maladaptive Emotion Regulation Strategies in Adolescents. Psychoneuroendocrinology. 2017;84:61-65.

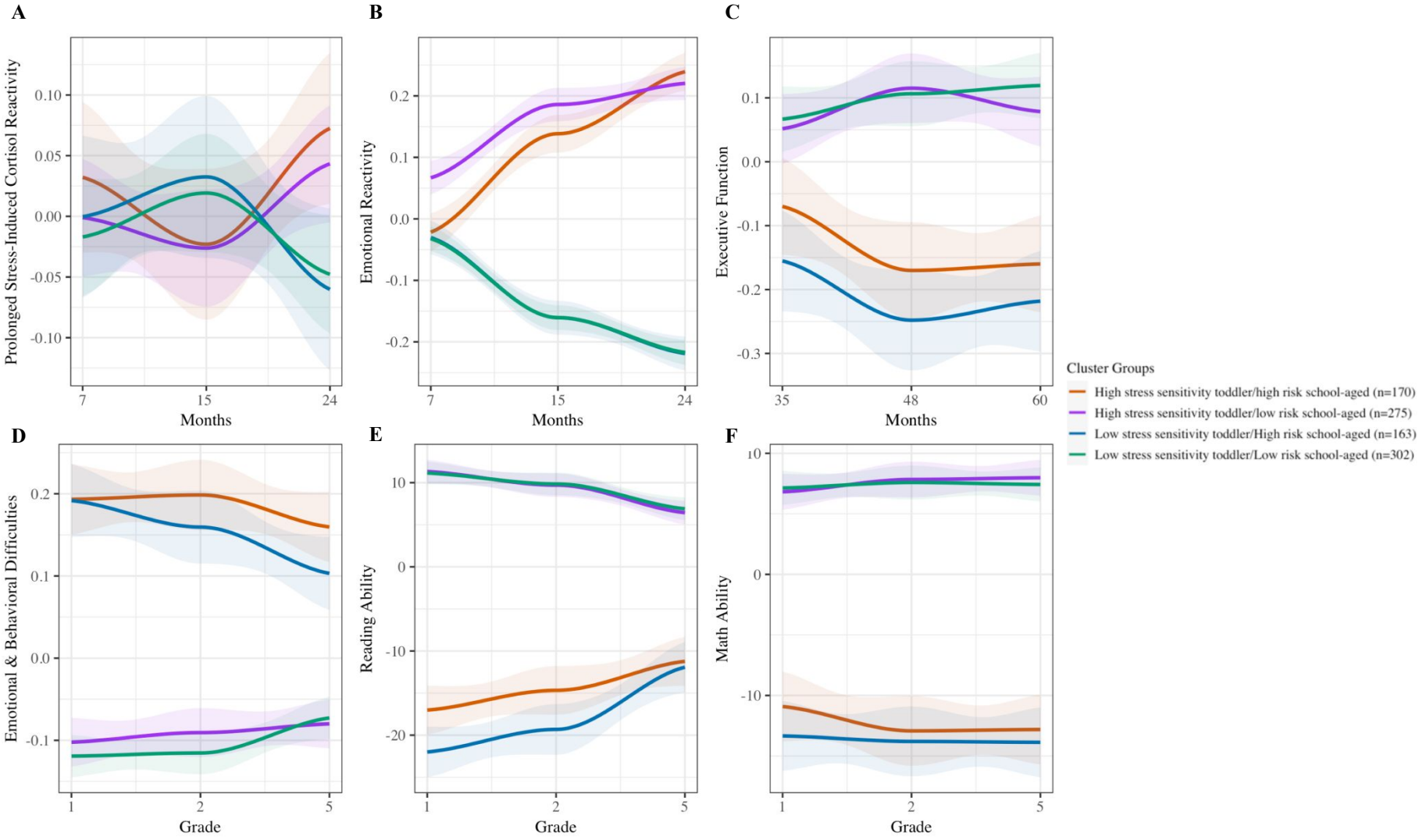

Figure 1. Trajectories of the cluster groups across time for each outcome. (A) Prolonged stress-induced cortisol at 7, 15 and 24 months by cluster group. (B) Emotional reactivity at 7, 15 and 24 months by cluster group. (C) Executive function at 36, 48 and 60 months by cluster group. (D) Emotional and

behavioral difficulties at grade 1, 2 and 5 by cluster group. (E) Reading ability at grade 1, 2 and 5 by cluster group. (F) Math ability at grade 1, 2 and 5 by cluster group.

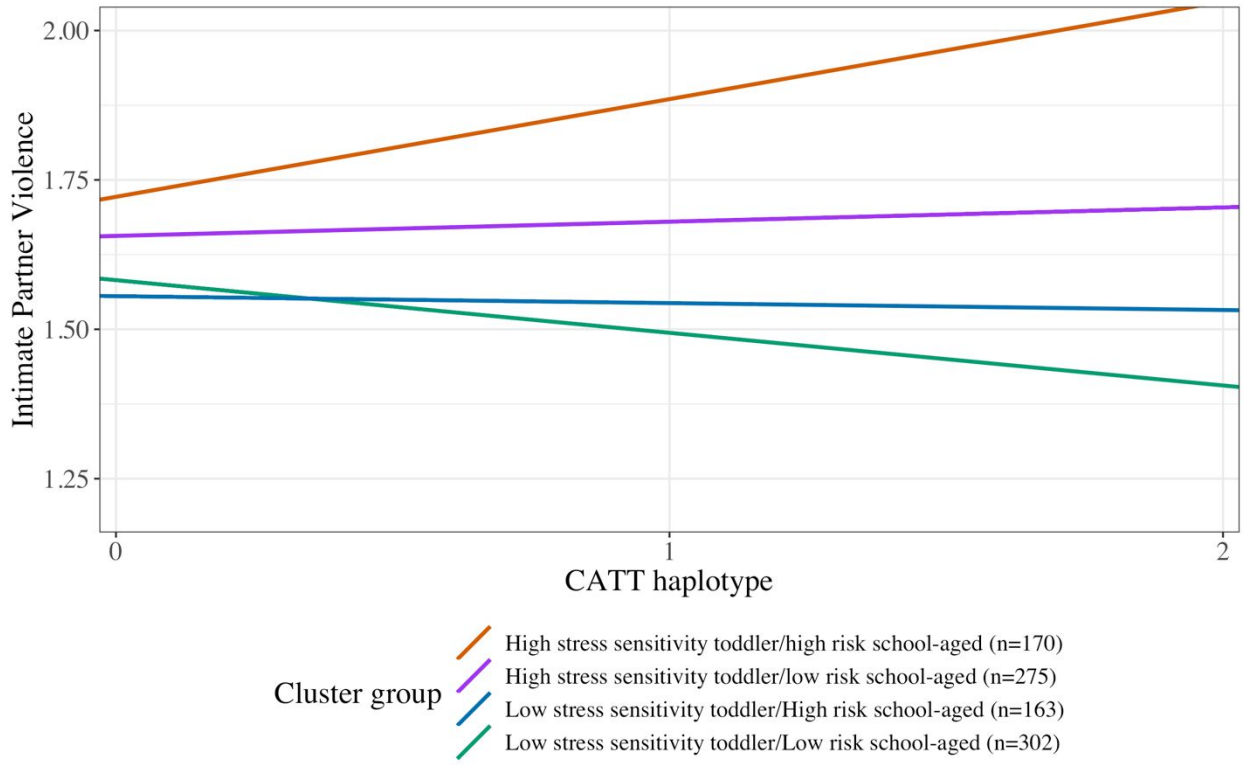

Figure 2. Representation of the interaction between IPV exposure and CATT haplotype (non-carrier=0, one CATT haplotype allele=1, and 2 CATT haplotype alleles=2) predicting the ranking of the cluster groups based on phenotype severity. Carriers of two copies of the *FKBP5* haplotype with high IPV were more likely to be in the high stress sensitivity toddler/high risk primary school cluster group at risk for deficits with executive function, emotional and behavioral problems and poor academic achievement (Cluster group BD) than the low stress sensitivity toddler/low risk primary school cluster group (Cluster group AC) (OR = 4·15, *p*= 0·031, 95% CI 0·99 – 21·12).

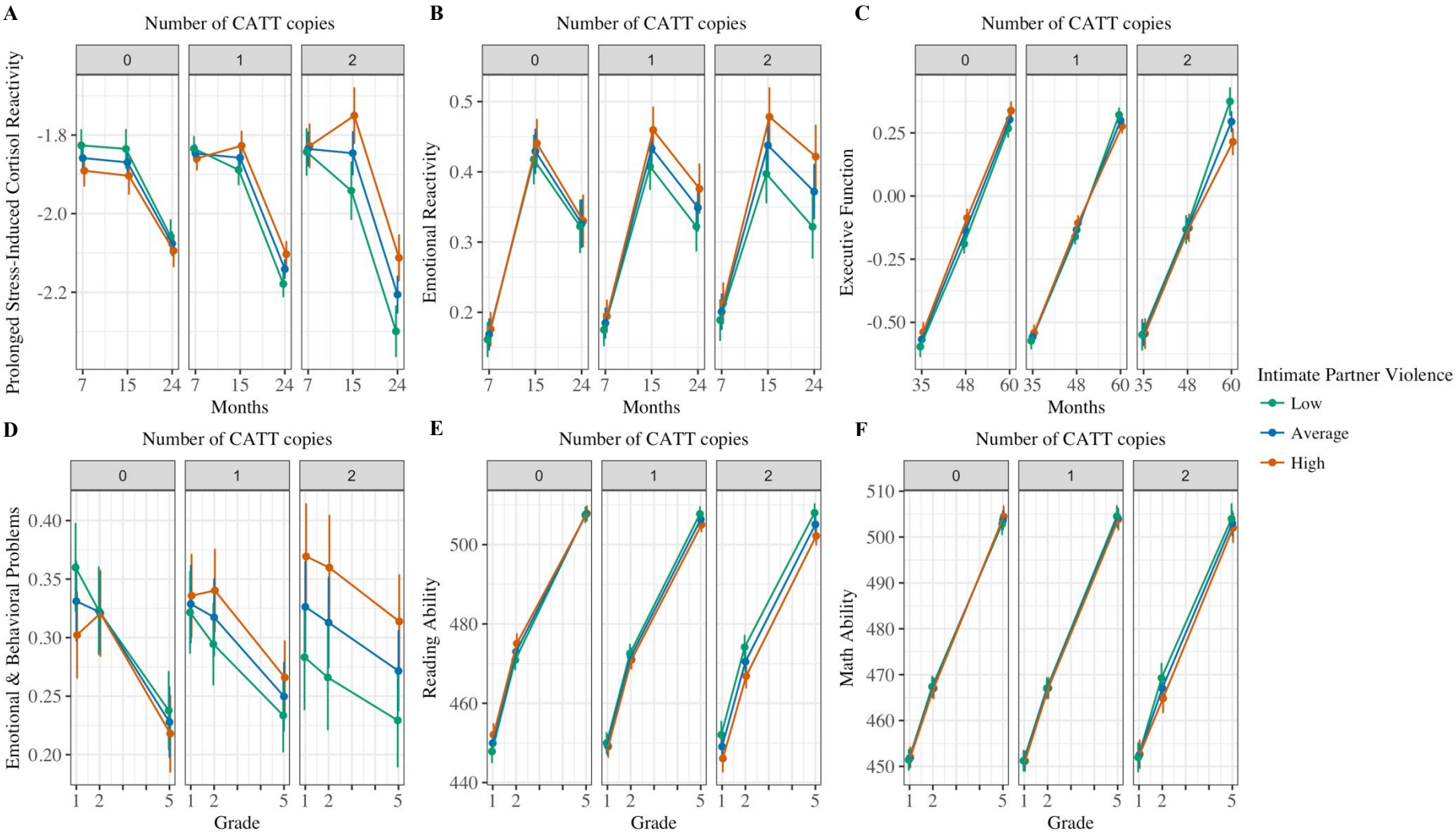

Figure 3. The trajectory of prolonged stress-induced cortisol reactivity (A), emotional reactivity (B), executive function (C), emotional and behavioral difficulties (D), and reading (E) and math (F) ability at average exposure to intimate partner violence (IPV) and exposure to intimate partner violence (IPV) during the child's first two years. Each panel depicts one standard error above and below the mean number of IPV exposure broken down by number of CATT haplotype copies (0 = no copies, 1 = 1 copy of the CATT haplotype, 2 = two copies of the CATT haplotype) across time. The estimated means of each outcomes have been adjusted for child sex, child race, child's state of residency, household chaos and cumulative risk (i.e., continuous

score based on family income-to-needs ratio, constant spouse/partner living in the home, maternal education, hours of employment, household density, occupational prestige, and neighborhood noise/safety measured at 7, 15, and 24 months postpartum). Additionally, prolonged stress induced cortisol reactivity scores have been adjusted for time of day the cortisol measures were taken, acetaminophen intake, and cortisol levels at baseline and 20 minutes following the stress paradigm. Mixed linear models revealed that the interaction significantly predicted prolonged stress-induced cortisol reactivity at 15 and 24 months ((b=0.10, se=0.05, *p*=0.035, ES = 0.13 and b=0.09, se=0.04, *p*=0.038, ES = 0.20, respectively), fear-elicited emotional reactivity at 24 months ((b=0.06, se=0.03, *p*=0.026, ES = 0.26), executive function at 60 months (b=-0.079, se=0.036, *p*=0.029, ES = 0.29), emotional and behavioral problems trajectory (b=0.048, se=0.021, *p*=0.020, ES = 0.77) and reading ability trajectory (b=-4.199, se=1.988, *p*=0.035, ES = 0.39).

Tables

Table 1.
Model comparison of the main, additive, and interactive effect of child CATT haplotype and IPV exposure on cluster group assignment. Each model has been adjusted for child sex, child state of residence, cumulative risk, household chaos, and ancestry markers.

Model	Log odds	SE	t-value	Comparison with the null model (*p*-value)
Model 1: Main effect of child CATT haplotype				
Child CATT haplotype	-0.170	0.090	-1.900	0.062
Model 2. Main effect of IPV				
IPV	-0.220	0.096	-2.300	0.021
Model 3. Additive effect				
CATT haplotype	-0.170	0.090	-1.800	0.013
IPV	-0.220	0.096	-2.300	
Model 4. Multiplicative interaction effect				
Child CATT haplotype	0.084	0.150	0.560	0.005
IPV	0.260	0.250	1.000	
Child CATT haplotype × IPV	-0.280	0.140	-2.100	

Note. IPV = intimate partner violence.