Association of maternal stress levels and the CRHR1 gene among mothers of a child with FXS

Emily Allen[1], Jessica Hunter[1]; Adriana Lori[1,3]; Debra Hamilton[1], Lisa Shubeck[1]; Ann Abramowitz[2]; Joseph F. Cubells[1,3]; and Stephanie Sherman[1]
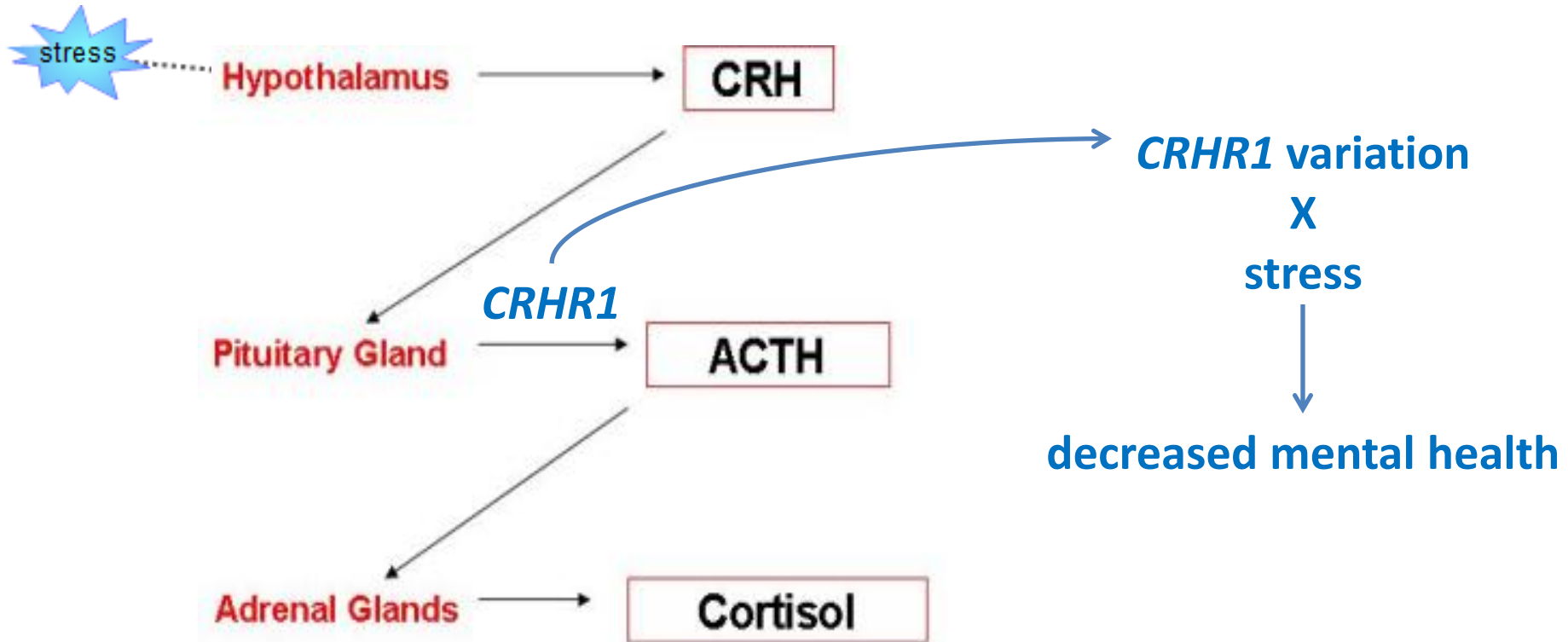
[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia; [2]Department of Clinical Psychology, Emory University, Atlanta, Georgia; [3]Department of Psychiatry and Behavioral

Sciences, Emory University School of Medicine, Atlanta, Georgia

HPA axis is responsible for the endogeneous stress response
CRH receptors are important mediators in the stress response

stress ---- Hypothalamus ——→ CRH

Pituitary Gland ——→ ACTH

Adrenal Glands ——→ Cortisol

CRHR1

CRHR1 variation
X
stress

↓

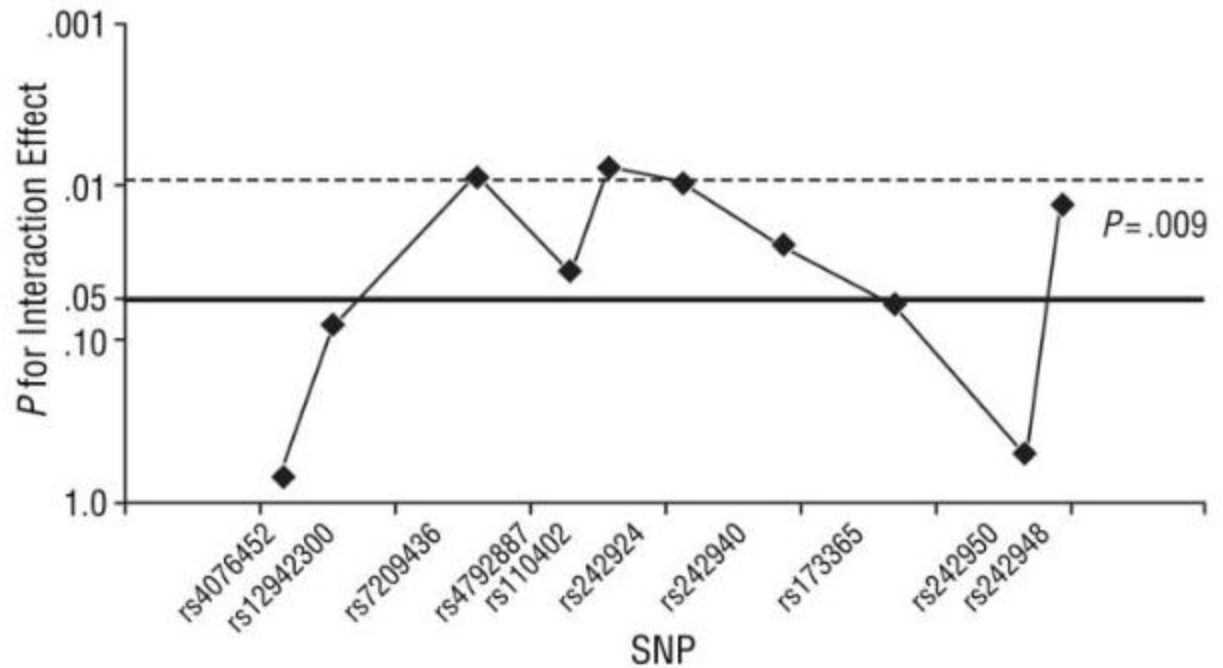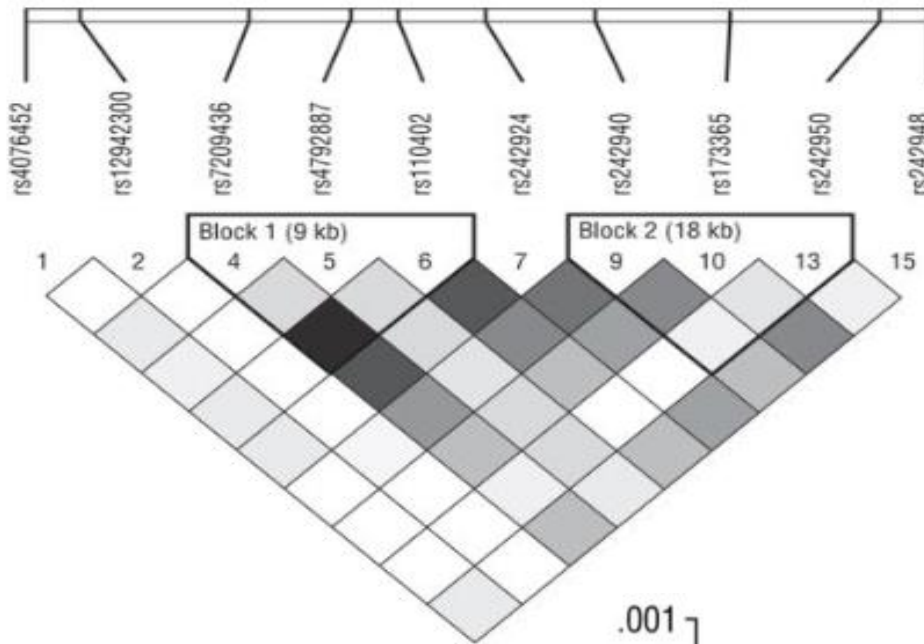**decreased mental health**

Bradley et al., 2008
Polanczyk et al., 2009
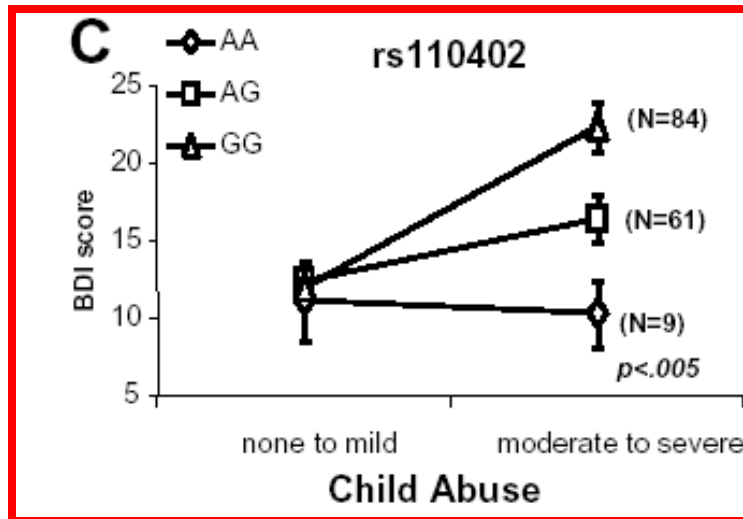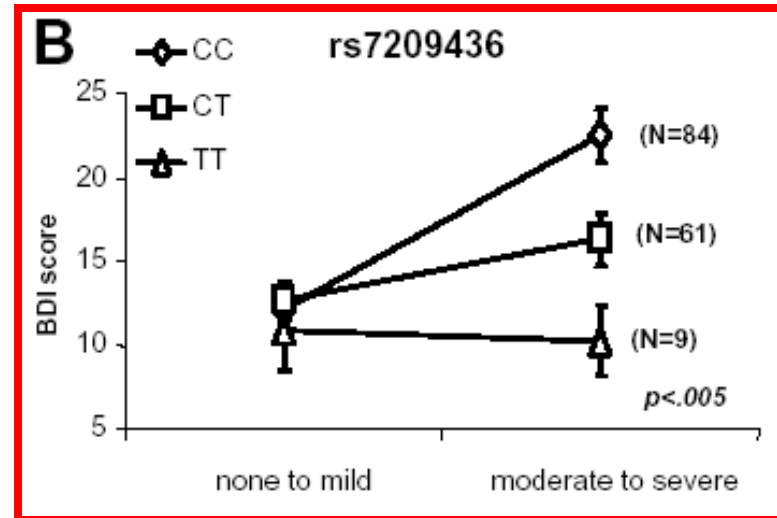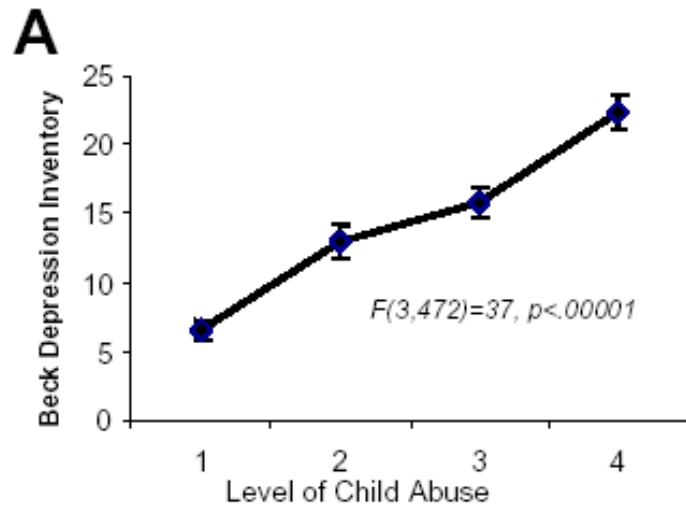
Influence of Child Abuse on Adult Depression

Moderation by the Corticotropin-Releasing Hormone Receptor Gene

Rebekah G. Bradley, PhD; Elisabeth B. Binder, MD, PhD; Michael P. Epstein, PhD; Yilang Tang, PhD; Hemu P. Nair, PhD; Wei Liu, PhD; Charles F. Gillespie, MD, PhD; Tiina Berg, PhD; Mark Evces, PhD; D. Jeffrey Newport, MD; Zachary N. Stowe, MD; Christine M. Heim, PhD; Charles B. Nemeroff, MD, PhD; Ann Schwartz, MD; Joseph F. Cubells, MD, PhD; Kerry J. Ressler, MD, PhD

- Gene X Environment association study of *CRHR1* gene and childhood stress

- Beck Depression Inventory (BDI)
  - 21 items to measure psychological and physical depression symptoms (2 weeks)
  - self-report (completed verbally to account for impaired literacy)

- Child Trauma Questionnaire (CTQ)
  - self report
  - sexual, physical, emotional abuse
  - cutoffs for none, mild, moderate, and severe

- Gene
  - 15 SNPs that span *CRHR1* gene

Polymorphisms moderated the effect of child abuse on risk for adult depression

Bradley et al. Arch Gen Psychiatry (2008); 65(2):190-200

A — *F(3,472)=37, p<.00001*

B rs7209436 — CC / CT / TT — (N=84), (N=61), (N=9) — *p<.005*

C rs110402 — AA / AG / GG — (N=84), (N=61), (N=9) — *p<.005*

D rs242924 — GG / GT / TT — (N=87), (N=60), (N=10) — *p<.005*

Bradley et al. Arch Gen Psychiatry (2008); 65(2):190-200

Depression and Anxiety Symptoms Among Women Who Carry the *FMR1* Premutation: Impact of Raising a Child With Fragile X Syndrome Is Moderated by *CRHR1* Polymorphisms

Jessica Ezzell Hunter,[1] Mary Leslie,[1] Gloria Novak,[1] Debra Hamilton,[1] Lisa Shubeck,[1] Krista Charen,[1] Ann Abramowitz,[2] Michael P. Epstein,[1] Adriana Lori,[1,3] Elisabeth Binder,[1,3] Joseph F. Cubells,[1,3] and Stephanie L. Sherman[1]*

Having a child with FXS moderates the effect of *CRHR1* SNP rs7209436 genotype to impact the SPAI social phobia score (p=0.0001).
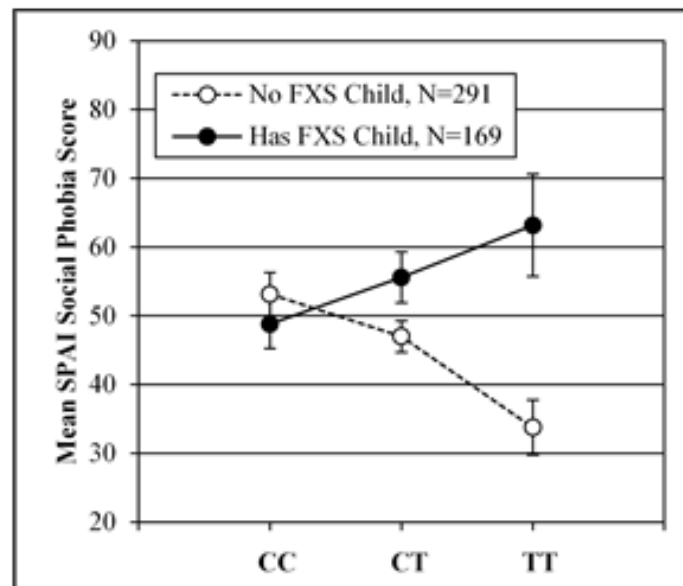
Measures Used:

Questionnaires

- **Social Phobia and Anxiety Inventory (SPAI)**
- Measures severity of distress associated with social interactions
- To distinguish pure social phobia from distress experienced in social situations due to panic disorder with agoraphobia, we calculated social phobia as: social phobia = social phobia subscale score - agoraphobia subscale score
- **Center for Epidemiologic Studies Depression Scale (CES-D)**
- Self-report questionnaire of depression symptoms in the past week
- **The State-Trait Anxiety Inventory (STAI)**
- State anxiety – current anxiety
- Trait anxiety – general anxiety susceptibility
- Self-report questionnaire
- **The Positive and Negative Affect Schedule (PANAS)**
- Self-report measure of psychological well being within the last year

		All	55-80 CGG repeats	81-100 CGG repeats	101-199 CGG repeats
N		**359**	**116**	**181**	**62**
Age at Test	Mean (SD)	40.3 (10.2)	40.5 (11.4)	40.6 (9.2)	39.2 (10.5)
Race	White AA/Other	89.5% 10.5%	90.5% 9.5%	87.3% 12.7%	93.5% 6.5%
Income	< $50,000 ≥ $50,000 Missing	28.5% 68.5% 3%	26.7% 69.8% 3.5%	29.8% 66.9% 3.3%	27.4% 71.0% 1.6%
Education	High school or less Some college +	15.3% 84.7%	14.7% 85.3%	14.9% 85.1%	17.7% 82.3%
FX child	Yes No Missing	54.6% 38.7% 6.7%	37.1% 45.7% 17.2%	61.3% 36.5% 2.2%	67.7% 32.3% 0 %

Is there a subset of premutation carriers who are more vulnerable to effects of stress?

Outcome = *CRHR1* + FXS child + (*CRHR1* x FXS child) + covariates

Outcome measures:

- Center for Epidemiologic Studies Depression Scale (CES-D)
- State-Trait Anxiety Inventory (STAI)
- Social Phobia and Anxiety Inventory (SPAI)
- Positive and Negative Affect Schedule (PANAS)

Covariates:

- Age
- Race/ethnicity
- Level of education
- *FMR1* repeat size group
- Household income

FMR1 Repeat Group	Low PM	Mid PM	High PM	All
N	116	181	62	359
% with FXS child	37.1%	61.3%	67.7%	54.6%

Rs7209436 (n=285)

Genotype	N
CC	96
CT	147
TT	42

Measure		Standardized β		
		FXS child	CRHR1	FXS child X CRHR1
CES	Depression	0.04	0.06	-0.06
STAI	State Anxiety	0.03	0.15	-0.11
	Trait Anxiety	0.11	0.11	-0.11
PANAS	Negative Affect	0.09	0.09	-0.02
	Positive Affect	0.03	-0.03	0.07
SPAI	Social Phobia	-0.12	-0.25*	0.32*
	Agoraphobia	0.08	0.04	-0.05

Rs110402 (n=279)

Genotype	N
GG	84
AG	148
AA	47

Measure		Standardized β		
		FXS child	CRHR1	FXS child X CRHR1
CES	Depression	0.04	0.03	-0.08
STAI	State Anxiety	0.04	0.11	-0.13
	Trait Anxiety	0.12	0.06	-0.12
PANAS	Negative Affect	0.09	0.04	-0.02
	Positive Affect	<0.01	-0.03	0.11
SPAI	Social Phobia	-0.12	-0.26*	0.29*
	Agoraphobia	0.05	-0.04	-0.01

Rs242924 (n=230)

Genotype	N
GG	78
GT	113
TT	39

Measure		Standardized β		
		FXS child	CRHR1	FXS child X CRHR1
CES	Depression	0.02	0.07	-0.03
STAI	State Anxiety	-0.04	0.09	-0.04
	Trait Anxiety	0.06	0.07	-0.05
PANAS	Negative Affect	0.05	0.04	-0.01
	Positive Affect	0.05	-0.04	0.05
SPAI	Social Phobia	-0.13	-0.21*	0.28*
	Agoraphobia	0.02	-0.01	0.05

Rs242940 (n=231)

Genotype	N
AA	71
AG	118
GG	42

Measure		Standardized β		
		FXS child	CRHR1	FXS child X CRHR1
CES	Depression	0.03	0.03	-0.05
STAI	State Anxiety	-0.05	0.01	-0.01
	Trait Anxiety	0.09	0.05	-0.10
PANAS	Negative Affect	0.08	0.03	-0.07
	Positive Affect	0.06	-0.01	0.06
SPAI	Social Phobia	-0.13	-0.26*	0.26*
	Agoraphobia	0.05	0.01	<0.01

Rs173365 (n=228)

Genotype	N
AA	46
AG	116
GG	66

Measure		Standardized β		
		FXS child	CRHR1	FXS child X CRHR1
CES	Depression	0.06	0.06	-0.08
STAI	State Anxiety	0.01	0.12	-0.09
	Trait Anxiety	0.13	0.08	-0.13
PANAS	Negative Affect	0.20	0.16	-0.22
	Positive Affect	0.04	-0.01	0.06
SPAI	Social Phobia	-0.01	-0.16	0.10
	Agoraphobia	0.17	0.12	-0.15

Is there a subset of premutation carriers who are more vulnerable to effects of stress?

Social phobia = *CRHR1* + **FXS child** + **(*CRHR1* x FXS child)** + *FMR1* **group + covariates**

- main effects not significant for any outcome
- interaction term significant only for SPAI social phobia
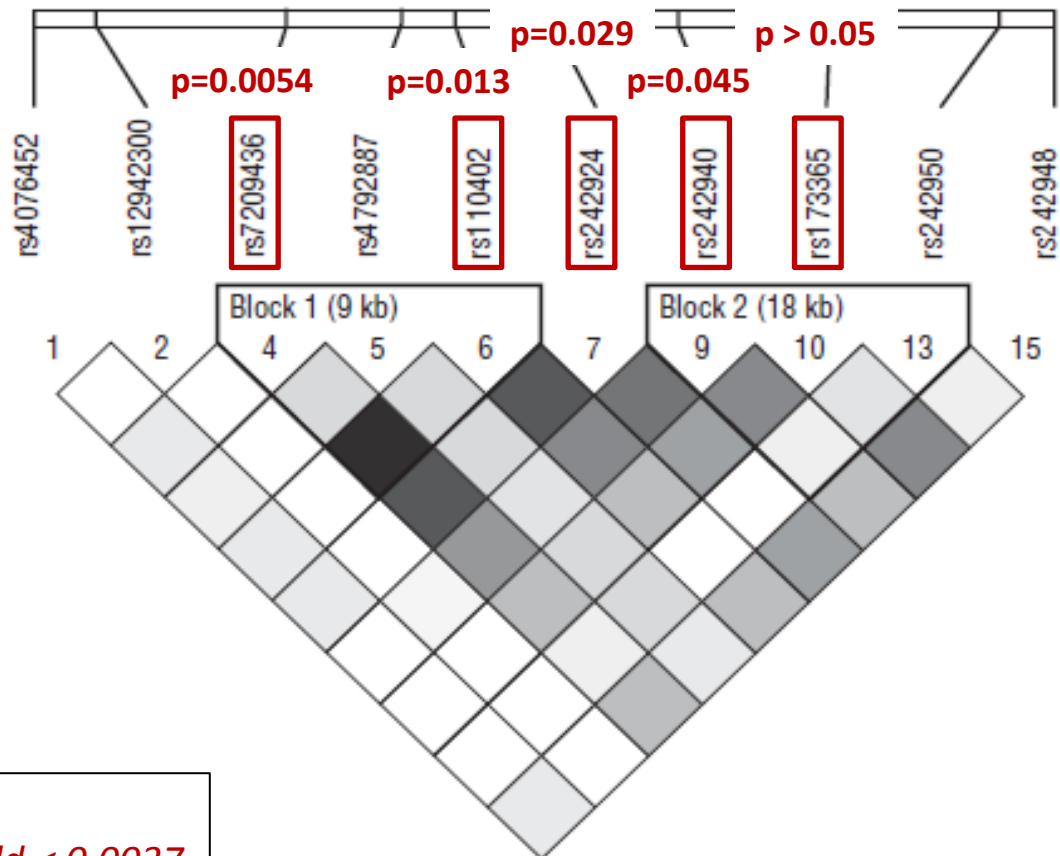
Adjusted p-value significance threshold < 0.0037

Measures Used:

- **Aberrant Behavior Checklist - Community (ABC-C)**

- Measures inappropriate and maladaptive behavior among individuals with intellectual disability

- 6 subscale scores refactored to be specific to behavior among children with FXS [Sansone et al. 2012 *J Autism Dev Disord 42(7): 1377-1392*]

- Irritability: disruptive and self-injurious behaviors

- Socially Unresponsive/Lethargic: social awareness and response

- Social Avoidance: gaze avoidance, social escape behaviors, and social anxiety

- Stereotypy: repetitive and recurring behavior

- Hyperactivity: elevated activity

- Inappropriate speech: repetitive and disruptive speech

- Total score = Sum of all 6 subscale scores

Questionnaire on Resources and Stress – Friedrich short version (QRS-F)

- Measures stress in families of children with disabilities

- 4 subscales

- Parent and Family Problems: stress related to child with disability on family

- Pessimism: parents' pessimistic beliefs regarding the child's future

- Child Characteristics: child's features associated with demands on parents

- Physical Incapacity: child's ability to perform a range of typical activities

- Total score = Parent and Family Problems subscale score + Pessimism subscale score [Honey et al. 2005 *Autism 9:246-255*]

Is there a subset of premutation carriers who are more vulnerable to effects of stress?

Outcome = *CRHR1* + Questionnaire + (*CRHR1* x Questionnaire) + covariates

Outcome measures:
- Social Phobia and Anxiety Inventory (SPAI)

Questionnaires:
- ABC
- QRS

Covariates:
- Age
- Race/ethnicity
- Level of education

- *FMR1* repeat size group
- Household income

FMR1 Repeat Group	All	Low PM	Mid PM	High PM
N	86	17	46	23
% with FXS child	100%	100%	100%	100%

Linear Regression Models
Outcome Variable: SPAI Social Phobia

Instrument	Subscale	R^2 Values		
		Subscale	rs7209436	SubscaleX rs7209436
ABC-C	Irritability	<0.01	<0.01	<0.01
	Socially Unresponsive / Lethargic	<0.01	0.01	<0.01
	Social Avoidance	<0.01	0.03	0.03
	Stereotypy	<0.01	0.01	0.02
	Hyperactivity	<0.01	0.02	0.03
	Inappropriate Speech	<0.01	0.03	0.04
	Total Score	<0.01	0.03	0.02
QRS-F	Parent and Family Problems	0.06	<0.01	<0.01
	Pessimism	<0.01	<0.01	<0.01
	Child Characteristics	0.03	<0.01	<0.01
	Physical Incapacity	<0.01	<0.01	<0.01
	Total Score	0.04	<0.01	<0.01

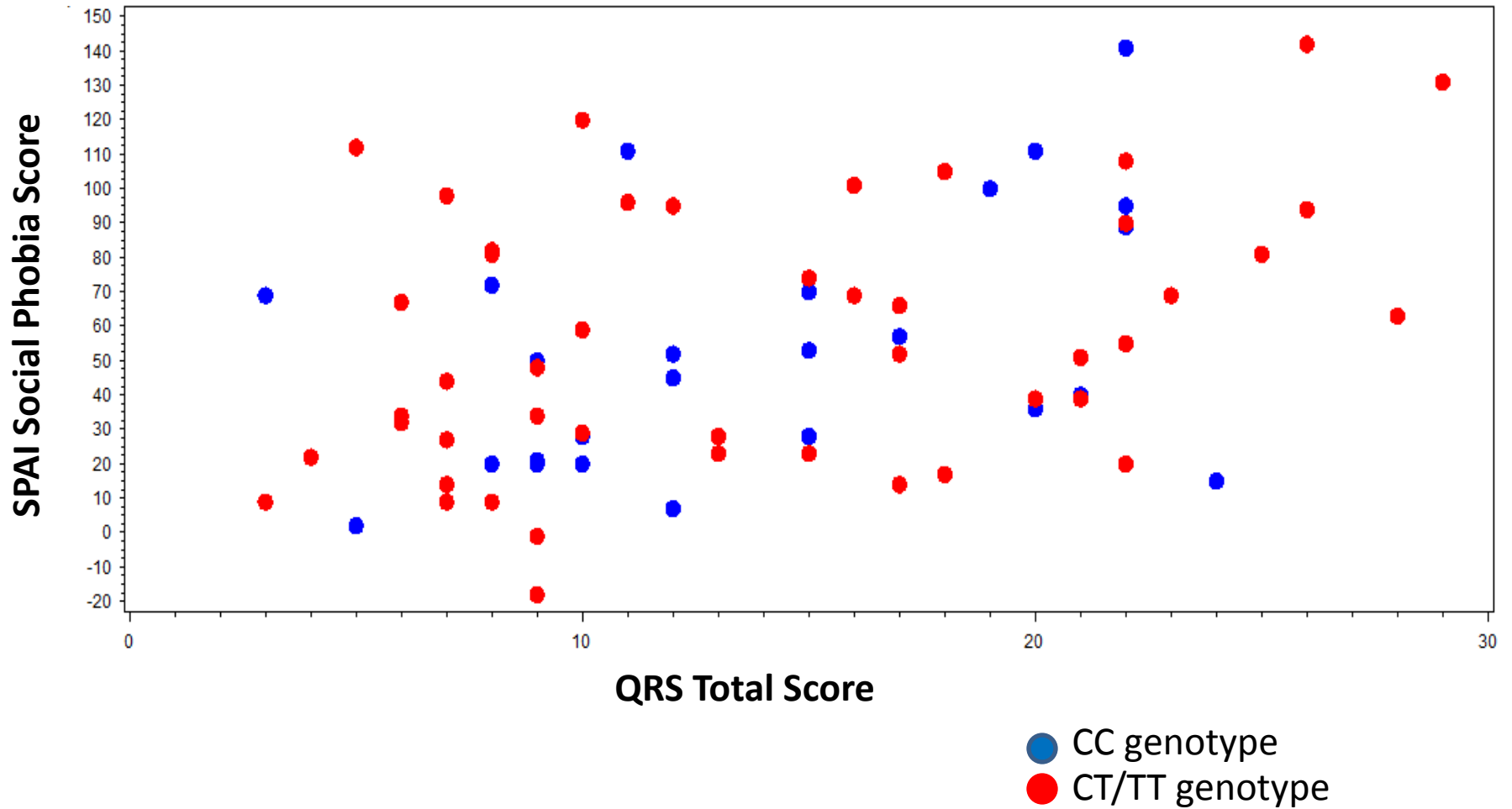# No significant interaction for CRHR1 genotype and child's behavior
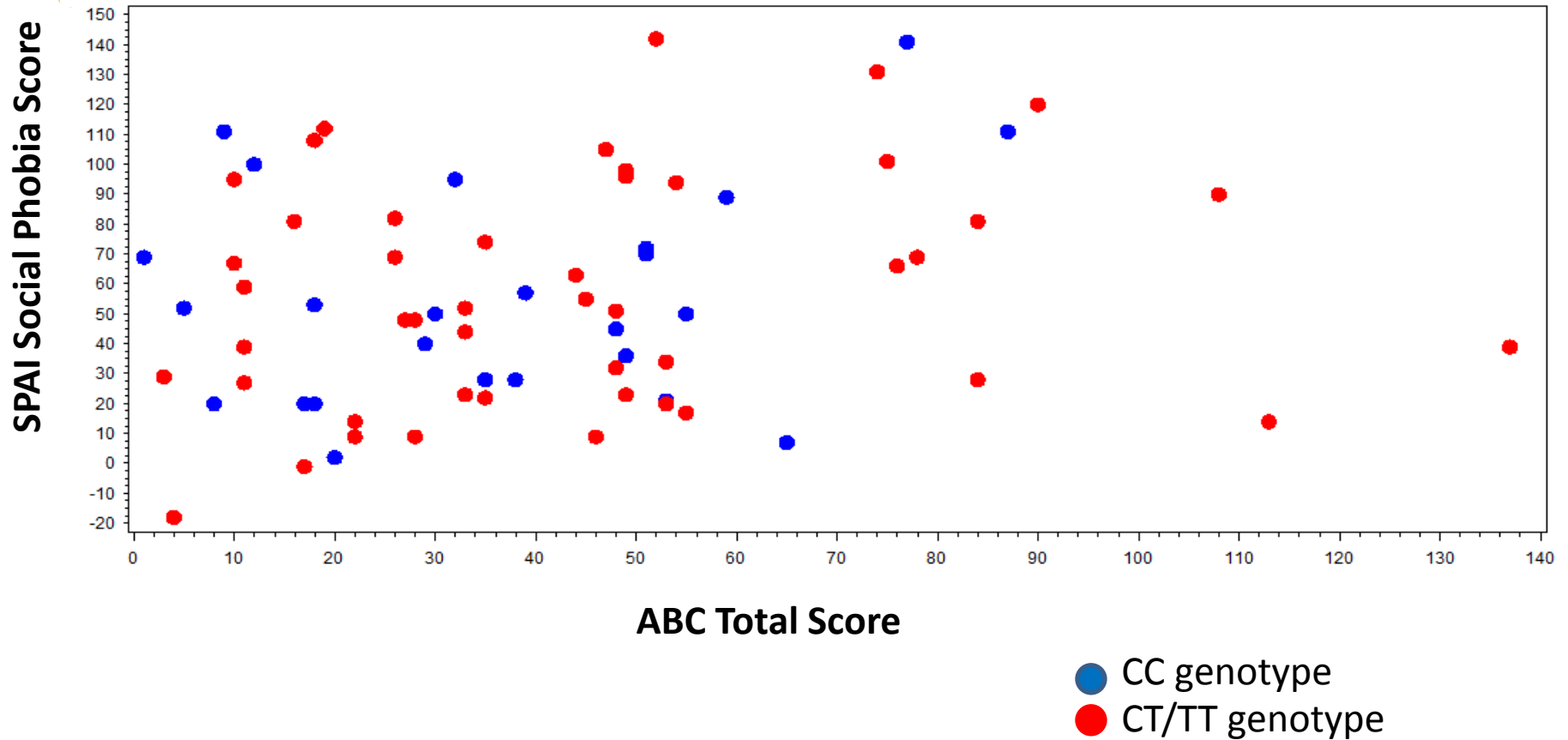
No significant interaction for CRHR1 genotype and family stress

Summary

- Having a child with FXS –
  - A significant gene by environment interaction was seen with CRHR1 genotypes and having a child with FXS
  - The effect was only detected on SPAI – specific to social phobia
- Could we identify what the stressor was?
  - Among mothers of a child with FXS, no relationships were identified for CRHR1 genotypes and family stress or behavior measures
  - Limitations –
    - Sample size
    - Wrong measures?

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This work could only be done with the significant efforts of families with FXAD

rs7209436

Instrument	Subscale	Genotypye		
		CC (N= 25)	CT (N=32)	TT (N=10)
ABC-C	Irritability	6.8	9.8	9.6
	Socially Unresponsive / Lethargic	3.9	9.2*	9.2
	Social Avoidance	1.9	4.6*	3.6
	Stereotypy	3.7	5.4	5.4
	Hyperactivity	12.6	13.6	17.6
	Inappropriate Speech	3.3	3.8	4.1
	Total Score	32.4	47.0	49.5
QRS-F	Parent and Family Problems	6.3	8.2	7.0
	Pessimism	6.4	7.2	6.0
	Child Characteristics	5.7	8.1	7.1
	Physical Incapacity	1.1	1.5	1.4
	Total Score	12.8	15.4	13.0

rs110402

Instrument	Subscale	Genotypye		
		GG (N= 22)	GA (N=33)	AA (N=10)
ABC-C	Irritability	7.1	9.7	9.4
	Socially Unresponsive / Lethargic	4.0	8.9	9.1
	Social Avoidance	2.0	4.4*	3.5
	Stereotypy	3.5	5.4	5.3
	Hyperactivity	13.4	13.0	17.6
	Inappropriate Speech	3.5	3.6	4.2
	Total Score	33.8	45.5	49.0
QRS-F	Parent and Family Problems	6.6	7.9	7.0
	Pessimism	6.6	7.1	6.0
	Child Characteristics	5.6	7.9	7.1
	Physical Incapacity	1.0	1.4	1.4
	Total Score	13.2	15.0	13.0