

**Mothers' Attachment is Linked to their Children's Anti-Inflammatory Gene Expression via
Maternal Warmth**

Supplemental Material

This online supplement contains additional analyses that test the links between mothers' attachment anxiety and attachment avoidance, youth-rated maternal warmth, and youths' glucocorticoid resistance of three asthma-related pro-inflammatory cytokines (i.e., IL-5, IL-13, and IFN- γ).

Method

Measures

Glucocorticoid resistance. We examined glucocorticoid resistance of three asthma-related pro-inflammatory cytokines (i.e., IL-5, IL-13, and IFN- γ). Up to a 6 mL peripheral blood sample was collected from each youth into Vacutainer Cell Preparation Tubes containing sodium heparin. Following a modified version of the protocol used by Miller et al. (2009), peripheral blood mononuclear cells (PBMCs) were isolated and resuspended in RPMI-1640 medium with HEPES supplemented with 10% Fetal Bovine Serum. PBMCs were treated with 25 ng/mL phorbol 12-myristate 13-acetate and 1 μ g/mL ionomycin calcium salt dissolved in DMSO and either 10 ng/mL hydrocortisone or vehicle control (1 μ l of 100% ethanol), then incubated at 37°C and 5% CO₂ for 48 hours. Cell suspensions were centrifuged, and supernatants were collected and frozen at -80°C. Concentrations of IL-5, IL-13, and IFN- γ were quantified by ELISA (R&D Systems, Minneapolis, MN). A standard curve was used to obtain absolute quantification of each analyte. Average concentration across duplicate experiments was calculated for each cytokine in each treatment condition. The coefficient of variation calculated across replicates was <15% for each analyte. For each individual and cytokine, glucocorticoid resistance was calculated by dividing the average concentration in the hydrocortisone-treated samples by that in the vehicle control treated samples. 112 youth had valid data for IL-5 glucocorticoid resistance, 111 youth had valid data for IL-13, and 104 youth had valid data for IFN- γ .

Statistical Analysis Strategy and Potential Covariates

The data analytic strategy for youths' glucocorticoid resistance of IL-5, IL-13, and IFN- γ as outcome variables was identical to the analytic strategy for youths' expression of the glucocorticoid receptor gene *NR3C1* described in the main text. Glucocorticoid resistance scores were winsorized at 3 standard deviations of the mean. Table S1 displays the bivariate correlations among study

variables. As in our primary analyses described in the main text, covariates that correlated with the outcome variables at a significance level of $p < .10$ or lower were included. For glucocorticoid resistance of IL-5, the covariates were mothers' education and mothers' relationship status. For glucocorticoid resistance of IL-13, the covariates were youths' medication use and youths' asthma severity diagnosis. For glucocorticoid resistance of IFN- γ , the covariates were only youths' gender.

Table S1
Correlations among Study Variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 Youth Gender ^a	—	-.02	-.11	.09	.03	.07	.17*	-.14	.004	.11	-.11	-.002	-.18*	.10	-.13	-.14	-.25**
2 Youth Ethnicity ^b		—	-.28**	-.25**	-.21*	.01	-.18*	-.30**	.14	-.17*	.20*	.20*	-.08	-.18*	.13	-.11	-.04
3 Mother Education ^c			—	.10	.14	-.08	-.04	.29**	-.05	-.14	-.04	-.05	.07	.05	-.18+	-.12	-.11
4 Mother Relationship Status ^d				—	-.01	.05	.06	.06	-.04	.07	-.18*	-.37**	.11	.16+	-.17+	-.04	-.07
5 Youth Medication Use ^e					—	.10	.13	.01	.01	.05	.05	.02	-.03	.08	.07	.25**	-.10
6 Youth Asthma Severity Diagnosis						—	.03	-.21*	.06	.15+	.08	.01	-.11	-.10	-.04	.16+	.11
7 Youth Age							—	.30**	.05	.07	-.03	.07	-.15+	-.04	-.06	-.01	.03
8 Mother Age								—	-.01	.01	-.01	.03	.10	-.12	-.05	-.08	-.01
9 Mother Depressive Symptoms									—	.62**	.52**	.41**	-.05	-.16+	.01	.05	-.01
10 Mother Neuroticism										—	.34**	.26**	-.12	-.16+	.05	.15	.05
11 Mother Attachment Anxiety											—	.66**	-.18*	-.25**	.07	.03	.04
12 Mother Attachment Avoidance												—	-.21*	-.26**	.19*	-.04	.01
13 Youth-Rated Maternal Warmth													—	.21*	-.05	.07	.08
14 Youth Expression of <i>NR3C1</i>														—	-.004	-.07	.13
15 Youth GR of IL-5															—	.56**	.03
16 Youth GR of IL-13																—	.06
17 Youth GR of IFN- γ																	—

Note. $N = 112$ youth (IL-5). $N = 111$ youth (IL-13). $N = 104$ youth (IFN- γ). $N = 132$ youth (all other variables).

GR = glucocorticoid resistance.

^a0 = male, 1 = female. ^b0 = White, 1 = non-White. ^c0 = less than one year of college, 1 = one year of college or more. ^d0 = not in relationship, 1 = in relationship. ^e0 = no, 1 = yes.

+ $p < .10$, * $p < .05$, ** $p < .01$

Results

Associations between Mothers' Attachment, Maternal Warmth, and Youths' Glucocorticoid Resistance

Glucocorticoid resistance of IL-5. Results of these analyses are displayed in Table S2. Mothers' greater attachment avoidance was linked with higher glucocorticoid resistance of IL-5 in offspring in an initial model without covariates (Model 1); however, this association did not remain robust when statistically adjusting for relevant covariates (Model 2). Mothers' attachment anxiety and youth-rated maternal warmth were not associated with youths' glucocorticoid resistance of IL-5 in any model.

Glucocorticoid resistance of IL-13. Results of these analyses are displayed in Table S3. No predictor variables of interest (i.e., mothers' attachment anxiety, attachment avoidance, and youth-rated maternal warmth) were linked with youths' glucocorticoid resistance of IL-13 in any model.

Glucocorticoid resistance of IFN- γ . Results of these analyses are displayed in Table S4. No predictor variables of interest (i.e., mothers' attachment anxiety, attachment avoidance, and youth-rated maternal warmth) were linked with youths' glucocorticoid resistance of IFN- γ in any model.

Table S2

Multiple Regression Associations between Mothers' Attachment Anxiety and Youth Glucocorticoid Resistance of IL-5

	Model 1		Model 2	
	Coeff(SE)	95% CI	Coeff(SE)	95% CI
<u>Analysis with Mother Attachment Anxiety</u>				
Mother Attachment Anxiety	.03(.04)	[-.05, .11]	.02(.04)	[-.06, .10]
Mother Education ^a			-.14(.08)+	[-.31, .02]
Mother Relationship Status ^b			-.14(.09)	[-.31, .03]
<u>Analysis with Mother Attachment Avoidance</u>				
Mother Attachment Avoidance	.08(.04)*	[.003, .16]	.07(.04)	[-.02, .15]
Mother Education ^a			-.15(.08)+	[-.31, .01]
Mother Relationship Status ^b			-.09(.09)	[-.27, .09]
<u>Analysis with Youth-Rated Maternal Warmth</u>				
Youth-Rated Maternal Warmth	-.02(.04)	[-.11, .07]	-.01(.04)	[-.10, .08]
Mother Education ^a			-.15(.08)+	[-.31, .02]
Mother Relationship Status ^b			-.14(.09)	[-.31, .03]

Note. $N = 112$ youth. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates. Coeff = coefficient; SE = standard error; CI = confidence interval. Continuous scores were calculated such that higher scores indicate greater standing on the variable (e.g., greater attachment anxiety). Continuous predictors and covariates were standardized.

^a0 = less than one year of college, 1 = one year of college or more. ^b0 = not in relationship, 1 = in relationship.

+ $p < .10$, * $p < .05$, ** $p < .01$

Table S3

Multiple Regression Associations between Mothers' Attachment Anxiety and Youth Glucocorticoid Resistance of IL-13

	Model 1		Model 2	
	Coeff(SE)	95% CI	Coeff(SE)	95% CI
<u>Analysis with Mother Attachment Anxiety</u>				
Mother Attachment Anxiety	.01(.03)	[-.04, .06]	.002(.02)	[-.05, .05]
Youth Medication Use ^a			.13(.05)*	[.03, .23]
Youth Asthma Severity Diagnosis			.04(.03)	[-.01, .09]
<u>Analysis with Mother Attachment Avoidance</u>				
Mother Attachment Avoidance	-.01(.03)	[-.06, .04]	-.01(.03)	[-.06, .04]
Youth Medication Use ^a			.13(.05)*	[.03, .23]
Youth Asthma Severity Diagnosis			.04(.03)	[-.01, .09]
<u>Analysis with Youth-Rated Maternal Warmth</u>				
Youth-Rated Maternal Warmth	.02(.03)	[-.03, .07]	.02(.03)	[-.03, .07]
Youth Medication Use ^a			.13(.05)*	[.03, .23]
Youth Asthma Severity Diagnosis			.04(.03)	[-.01, .09]

Note. $N = 111$ youth. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates. Coeff = coefficient; SE = standard error; CI = confidence interval. Continuous scores were calculated such that higher scores indicate greater standing on the variable (e.g., greater attachment anxiety). Continuous predictors and covariates were standardized.

^a0 = no, 1 = yes.

+ $p < .10$, * $p < .05$, ** $p < .01$

Table S4

Multiple Regression Associations between Mothers' Attachment Anxiety and Youth Glucocorticoid Resistance of IFN- γ

	Model 1		Model 2	
	Coeff(SE)	95% CI	Coeff(SE)	95% CI
<u>Analysis with Mother Attachment Anxiety</u>				
Mother Attachment Anxiety	.06(.12)	[-.19, .30]	.02(.12)	[-.23, .26]
Youth Gender ^a			-.64(.25)*	[-1.14, -.14]
<u>Analysis with Mother Attachment Avoidance</u>				
Mother Attachment Avoidance	.01(.13)	[-.24, .27]	-.002(.13)	[-.25, .25]
Youth Gender ^a			-.64(.25)*	[-1.14, -.15]
<u>Analysis with Youth-Rated Maternal Warmth</u>				
Youth-Rated Maternal Warmth	.10(.13)	[-.16, .36]	.06(.13)	[-.20, .31]
Youth Gender ^a			-.63(.25)*	[-1.13, -.13]

Note. $N = 104$ youth. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates. Coeff = coefficient; SE = standard error; CI = confidence interval. Continuous scores were calculated such that higher scores indicate greater standing on the variable (e.g., greater attachment anxiety).

Continuous predictors and covariates were standardized.

^a0 = male, 1 = female.

+ $p < .10$, * $p < .05$, ** $p < .01$

Indirect Associations of Mothers' Attachment on Youths' Glucocorticoid Resistance via Maternal Warmth

As in our primary analyses described in the main text, we used the PROCESS macro for SPSS (Hayes, 2013) to estimate the indirect associations of mothers' attachment anxiety and avoidance with youths' glucocorticoid resistance via youth-rated maternal warmth. Bias-corrected bootstrap confidence intervals for indirect effects were estimated based on 10,000 bootstrap samples.

Glucocorticoid resistance of IL-5. As displayed in Figure S1, Panel A, analyses indicated that there were no significant indirect associations of mothers' attachment anxiety through maternal warmth with youths' glucocorticoid resistance of IL-5. Turning to Figure S1, Panel B, analyses indicated that that there were no significant indirect associations of mothers' attachment avoidance through maternal warmth with youths' glucocorticoid resistance of IL-5.

Glucocorticoid resistance of IL-13. As displayed in Figure S2, Panel A, analyses indicated that there were no significant indirect associations of mothers' attachment anxiety through maternal warmth with youths' glucocorticoid resistance of IL-13. Turning to Figure S2, Panel B, analyses indicated that that there were no significant indirect associations of mothers' attachment avoidance through maternal warmth with youths' glucocorticoid resistance of IL-13.

Glucocorticoid resistance of IFN- γ . As displayed in Figure S3, Panel A, analyses indicated that there were no significant indirect associations of mothers' attachment anxiety through maternal warmth with youths' glucocorticoid resistance of IFN- γ . Turning to Figure S3, Panel B, analyses indicated that that there were no significant indirect associations of mothers' attachment avoidance through maternal warmth with youths' glucocorticoid resistance of IFN- γ .

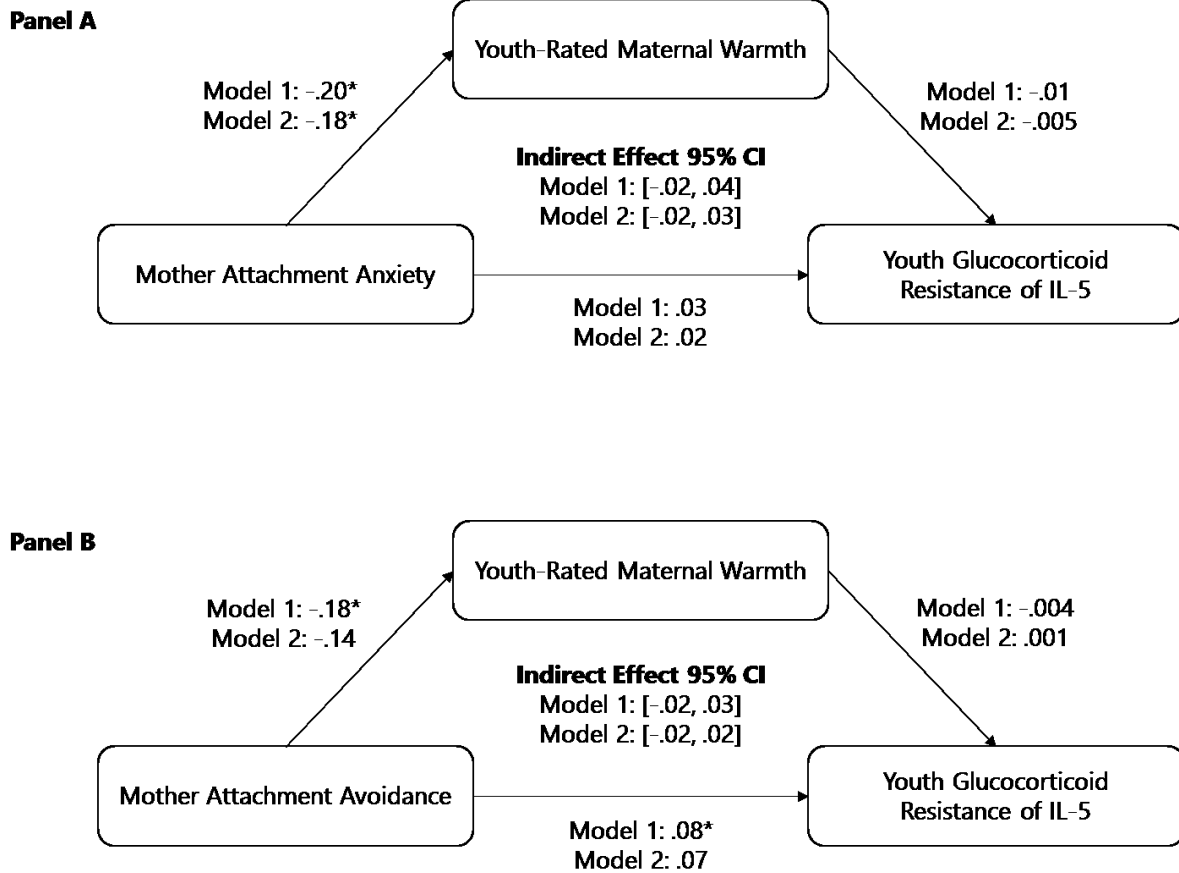


Figure S1. $N = 112$ youth. Direct and indirect associations between mother romantic attachment anxiety (Panel A), mother romantic attachment avoidance (Panel B), youth-rated maternal warmth, and youth glucocorticoid resistance of IL-5. CI = confidence interval. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates.

* $p < .05$

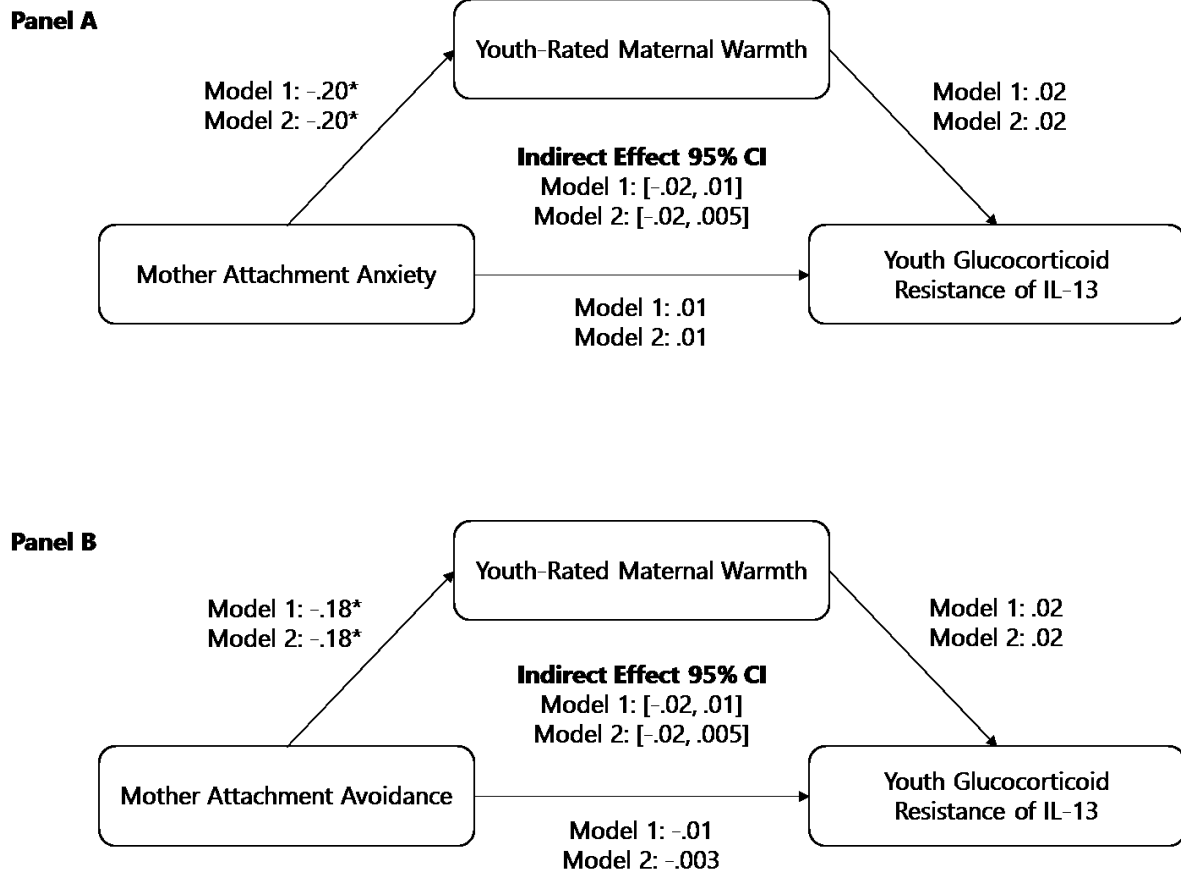


Figure S2. $N = 111$ youth. Direct and indirect associations between mother romantic attachment anxiety (Panel A), mother romantic attachment avoidance (Panel B), youth-rated maternal warmth, and youth glucocorticoid resistance of IL-13. CI = confidence interval. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates.

* $p < .05$

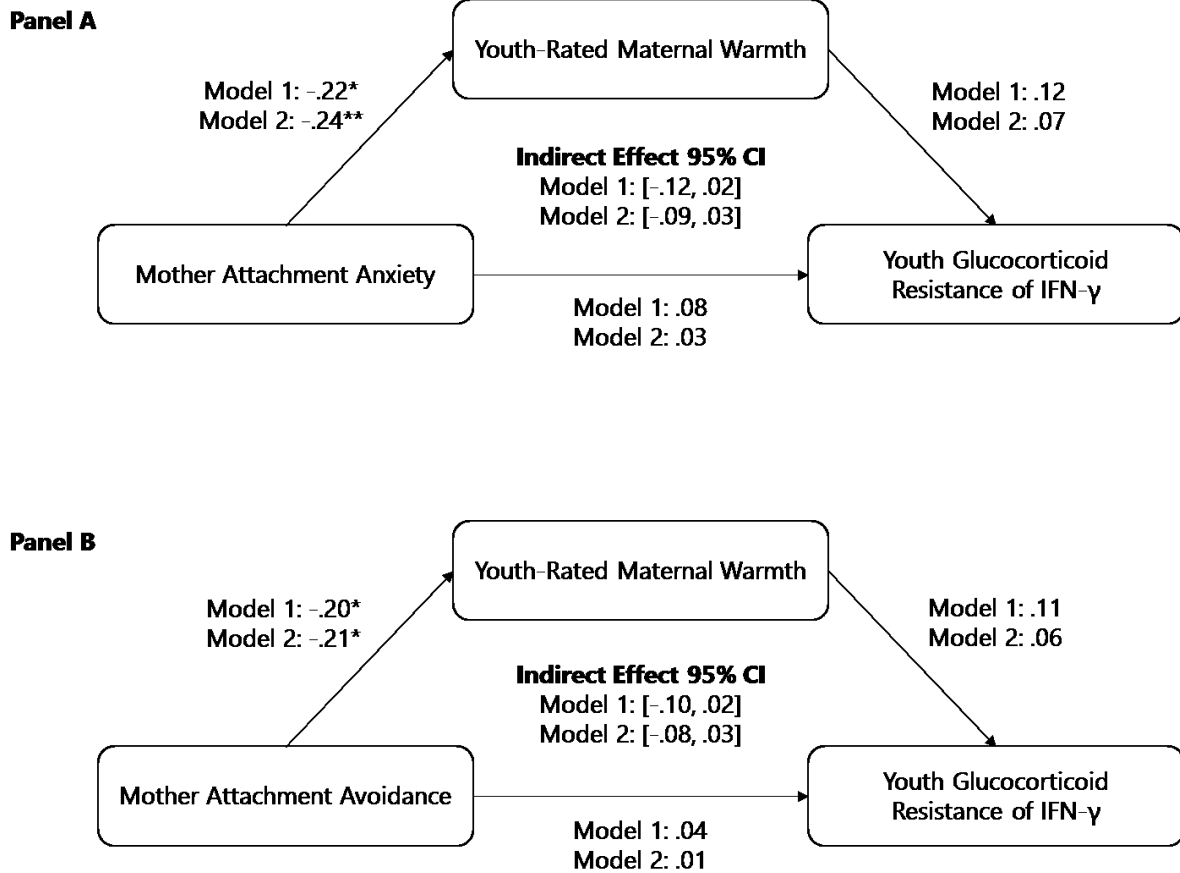


Figure S3. $N = 104$ youth. Direct and indirect associations between mother romantic attachment anxiety (Panel A), mother romantic attachment avoidance (Panel B), youth-rated maternal warmth, and youth glucocorticoid resistance of IFN- γ . CI = confidence interval. Model 1 = original analysis with no covariates. Model 2 = analysis with covariates.

* $p < .05$, ** $p < .01$

Discussion

Although the results for glucocorticoid resistance were not statistically significant, we believe our key finding in the main text regarding glucocorticoid receptor gene expression is still important and informative. In our supplemental analyses, we were able to test glucocorticoid resistance for IL-5, IL-13, and IFN- γ , which represent only a small sample of immune markers that could be related to glucocorticoid resistance. It may be that mothers' attachment and warmth is linked to their children's glucocorticoid resistance as measured by other inflammatory markers that we were unable to test in the present study. Additionally, our assays were conducted *in vitro*, and it may be that investigating these links *in vivo* is more effective. Future studies should aim to disentangle the links between parents' attachment, warmth, and their children's glucocorticoid resistance.