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Parental Depressive Symptoms Potentiate the Effect of Youth Negative Mood Symptoms on Gene Expression in Children with Asthma

Erika M. Manczak[1] · Bryn Dougherty[2] · Edith Chen[3]

Abstract

Depressive symptoms in parents and in youths have been found to relate to disease comorbidity processes in children, including greater disease-related impairment and poorer clinical outcomes. The current study sought to assess whether coming from a family characterized by more depressive symptoms on average would potentiate the effects of changes in youths' own negative mood on the expression of two receptor genes relevant to asthma that are the primary targets of asthma medication, such that the combination of low child negative mood in the context of greater parental depressive symptoms would relate to the lowest levels of gene expression. One-hundred-twenty youths with diagnosed asthma and their parents participated every 6 months for 2 years. Parents reported on their depressive symptoms, children reported negative mood symptoms, and youths completed blood draws from which expression of Glucocorticoid Receptor (GR) and Beta2 Adrenergic Receptor (β_2-AR) genes was extracted. Multilevel linear modeling revealed significant interactions between average levels of parental depressive symptoms and changes in youths' negative mood symptoms predicting gene expression, such that youths expressed significantly less GR and β_2-AR during times when they experienced more negative mood symptoms, but this was only true if they came from families with higher levels of average parental depressive symptoms. The current study identifies novel and biologically-proximal molecular signaling patterns that connect depressive symptoms to pediatric asthma while also highlighting the important role of family environment for biological processes that may operate within depression comorbidity.

Keywords Depression · Parents-child relationships · Asthma · Molecular genetics

Symptoms of depression in children not only relate to poorer psychosocial functioning, but also predict worse physical health outcomes (Pinquart and Shen 2011). For example, depression at age 15 has been shown to prospectively predict worse self- and interviewer-perceived health, higher health care utilization, and greater work impairment due to physical health at age 20 (Keenan-Miller et al. 2007). Not only do children with more depressive symptoms report higher levels of somatic complaints, broadly (Egger et al. 1999; Pine et al. 1996), but comorbid depression in the context of a pediatric illness is reliably predictive of poorer disease-specific functioning. For instance, meta-analytic work finds that adolescent depression is associated with greater treatment nonadherence in youths with diabetes, which in turn relates to worse clinical disease outcomes (Gonzalez et al. 2008). Depressive symptoms are similarly associated with lower quality of life in individuals with pediatric inflammatory bowel disease (Herzer et al. 2011) and with chronic pediatric pain (Kashikar-Zuck et al. 2001). To better understand the full impact of depressive symptoms on the lives of youths, it is therefore critical for research to identify psychological and biological processes that underlie comorbidity between depression and pediatric chronic illness.

Although functional impairment associated with depressive symptoms is evident across a variety of diseases, pediatric asthma provides a particularly relevant disease context in which to examine mechanisms and consequences of depression

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comorbidity. Asthma is a chronic respiratory disorder that is characterized by inflammation and obstruction of the airways. As a disease, it is especially appropriate to examine in relation to psychological comorbidity because it is one of the most common chronic illnesses of childhood (National Center for Health Statistics 2013), is well understood (Wright 2011), has previously shown robust connections to psychological phenomena like stress (Bloomberg and Chen 2005), and operates on a relatively fast time-scale that is conducive to more temporally-precise assessments (Skoner 2002). Moreover, children with asthma have a two-fold higher prevalence of depressive/anxiety symptoms compared to children without asthma (Katon et al. 2007). In turn, depressive symptoms are associated with more frequent hospital visits (Ahmedani et al. 2013), greater medication non-compliance (DiMatteo et al. 2000), greater social and educational impairment (Gutstadt et al. 1989), and increased risk of dying from asthma-related causes (Strunk et al. 1985).

To understand how these associations between asthma and depressive symptoms occur biologically, an examination of molecular signaling pathways may provide insight into important and proximal processes. Briefly, although an individual's DNA sequence serves as a blueprint that informs downstream cell functioning, not all genes are active in any given moment. Rather, the process of gene expression entails the synthesis of RNA molecules, which are then translated into proteins that are used to create structures within cells and to carryout functions by cells. Importantly, the transcription of many genes are believed to be sensitive to social and emotional conditions, such that expression is increased or decreased in response to experiential input (Cole et al. 2007).

Of particular relevance to the pathophysiology of asthma are glucocorticoid receptors (GR) and β_2-adrenegeric receptors (β_2-AR). GR is critical to anti-inflammatory signaling cascades that regulate the activity of immune cells, antibody generation, and cytokine production, whereas β_2-AR binds with epinephrine, which, among other functions, influences bronchodilation and smooth muscle functioning. These receptors are also the primary targets of medications for asthma. Corticosteroids, which act on GR, typically serve as controller medications for asthma, while β_2-AR-agonists are typically prescribed as rescue medications to be administered during acute exacerbations and act on β_2-AR (Busse and Lemanske 2001). Individuals who express less of either receptor would be expected to show greater asthma symptoms and less responsivity to asthma medications. Importantly, previous research suggests that the expression of these receptors is sensitive to psychological experiences. For example, Miller and Chen (2006) demonstrated that children with asthma who experienced both acute and chronic stress showed 5.5 times less GR mRNA and 9.5 times less β_2-AR mRNA than those without similar stress exposure. Specific to depression, biologically-plausible explanations for connections between mental health and GR and β_2-AR expression include heightened activation of stress-responses systems

and inflammatory processes that are shared by asthma and depression (Van Lieshout et al. 2009), such as those that account for well-documented associations between depression and cortisol secretion profiles in medically-healthy populations (Adam et al. 2010). Another possibility is that behavioral changes associated with depression, such as less adherence to medication, may effect expression of GR and β_2-AR (Grenard et al. 2011). It is important to note that these genes, particularly GR, have also been implicated in mood disorders (for a review, see Pariante and Lightman 2008). Thus, it is also possible that associations between depression and GR expression are due to reverse directionality or are bi-directional.

Although children's depressive symptoms, such as negative mood, may directly predict gene expression in pediatric asthma, elements of the family context may also contribute to youths' gene expression profiles by modifying the effect of children's psychopathology symptoms. During childhood, parents can play a critical role in preventing, reducing, or overcoming depressive problems, such that parenting interventions are commonly utilized in the treatment of youth depression (Compas et al. 2010). In turn, these dynamics may also moderate disease processes that are affected by depression. This possibility is supported by substantial research documenting connections between family functioning and youth asthma. For example, Marin et al. (2009) found that the effects of acute stress on the production of asthma-related cytokines among children with pediatric asthma were magnified for children who experienced high levels of chronic family stress, but were buffered for children with low levels of family stress. Similarly, a negative family emotional climate has been shown to predict youths' asthma severity by affecting children's depressive symptoms and potentiating emotional triggering of asthma (Wood et al. 2006).

One powerful contributor to problematic family environments is parental depression. Indeed, mothers with depression reliably demonstrate more negative and coercive behaviors, greater disengagement, and lower levels of warmth and positivity toward their children than non-depressed mothers, and these impairments endure even once depression recedes (Lovejoy et al. 2000; Thomas et al. 2014). For this reason, it may be useful to consider exposure to parental depressive symptoms in the aggregate across a given period of time, and its effects on children's functioning. Moreover, parental depression confers multifactorial risk, both through a presence of aversive parenting behaviors as well as through an absence of parental support (Sellers et al. 2014). For example, even if parents overcome a tendency toward punitive parenting when depressed, children may still face a less supportive home environment that fails to help them regulate their own mood difficulties. This difficulty for depressed parents to act as a buffer is supported by a longitudinal study comparing children from mothers with affective disorders to those from healthy mothers, which found that children with symptomatic mothers showed more subsequent depressive symptoms in response to

stressful experiences than children who experienced stressful events but who had mothers without depressive symptoms (Hammen et al. 1991).

For children with chronic illnesses, these environmental features may have repercussions for youths' biological functioning. In a sample of children with and without asthma, higher levels of parental depressive symptoms predicted increases in children's eosinophil cationic protein—an asthma-relevant inflammatory marker—over a six-month period, and this was not accounted for by children's own depressive symptoms (Wolf et al. 2008). Parental depressive symptoms have also been shown to predict more hospitalizations (Lange et al. 2011), greater use of the emergency department (Bartlett et al. 2001), and more difficulties with using inhaler medication properly (Bartlett et al. 2004) among children with asthma. The extent to which parental depressive symptoms would interact with children's own affective problems to predict asthma-relevant gene expression remains unknown, however.

To address this gap, the current study utilized five waves of longitudinal data across a two-year period to test whether average parental depressive symptoms would moderate the effect of changes in youths' negative mood symptoms on the expression of GR and β_2-AR genes in the leukocytes of youths with physician-diagnosed asthma. These genes were chosen because they are the primary targets of asthma medication and thus represent biologically-proximal mediators of comorbidity. We hypothesized that children from family contexts generally characterized by higher levels of parental depressive symptoms would demonstrate gene expression profiles that were more sensitive to changes in their own negative mood symptoms, predicting that these youths would show significantly less expression of GR and of β_2-AR during periods when they experienced more negative mood symptoms than average, whereas children from families with lower average parental depressive symptoms would not show differences in their gene expression during times of greater negative mood symptoms. We anticipated that these associations would emerge above and beyond demographic features of parents and youths (including gender, socioeconomic status, and age), leukocyte distributions, asthma medication use, and asthma severity. We also tested a reverse-directionality model in which changes in gene expression (and their interaction with average parental depressive symptoms) predicted youth depressive symptoms.

Methods

Participants

Youths ages 9- to 18-years-old with physician-diagnosed asthma were recruited to participate with one parent, as part of a larger study, through newspaper and community advertisements and through asthma clinics in a large North American

city. Youths and parents were required to be fluent in English and youths were required to have received a diagnosis of asthma from a physician, to be free of acute respiratory illnesses at the time of their first visit, to have been free of oral steroids for at least 2 weeks preceding their baseline visit, and to be free of medication for chronic disorders besides asthma. One-hundred-twenty youths and their parents met these criteria and enrolled in the larger study. Reflecting gender differences in the prevalence of pediatric asthma (Almqvist et al. 2007), 67% of youths in the current sample were male. Sixty-three percent of children identified as White and were on average 12.61 years old ($SD = 2.63$). Eighty-three percent of parents were female and average family income was in the $50,000–74,999 Canadian dollar range.

Procedure

Parents provided written consent and youths provided written assent, overseen by the Institutional Review Board of the University of British Columbia. Youths and parents came to the lab for a baseline assessment, during which participants reported on their demographic information and parents completed a questionnaire about their depressive symptoms. Six months later, parents and youths returned to the lab, during which parents again reported their depressive symptoms and youths completed a measure of negative mood, reported on their asthma medication use, and underwent a blood draw. The assessments of parent symptoms, youth mood symptoms, medication, and blood were repeated every six months for an additional year and a half, resulting in biological data at up to four time points per youth plus one additional baseline assessment of parent symptoms (a two year period in total). The majority of dyads (75.5%) completed all five possible visits and 94.5% completed two or more visits. Analyses were based on all available data at each time-point. There were no significances differences on any study variables between families who provided complete data at all time points and those with any missing data.

Measures

Parent Depressive Symptoms At each of the 5 visits, parents completed the Center for Epidemiological Studies—Depression Scale (Radloff 1977), a widely-used 20-item questionnaire that asks participants to rate on a 0–3 scale how often they experienced various depressive symptoms during the past week, with higher scores indicating more depression symptomatology (Cronbach's $\alpha = 0.90$ in current sample). The reliability and validity of this measure have been robustly demonstrated in both community and clinical samples (Lewinsohn et al. 1997; Shinar et al. 1986). In the present study, we were interested in how living in a family environment generally characterized by different levels of parental depressive symptoms would moderate the effects of

experiencing negative moods on children's gene expression, guided by research documenting the enduring and widespread effects of current as well as previous parental depressive episodes (Lovejoy et al. 2000). To that end, we treated parental depressive symptoms as a largely stable factor, averaging CES-D scores across all available visits. This decision was supported by the significant correlations between scores at every visit and the high reliability of the total score across the five visits (Cronbach's $\alpha = 0.83$). Parental depressive symptoms were measured as a continuous variable, but for interpretation purposes, follow-up analyses plotted associations at the 25th percentile ("low parental depressive symptoms") and 75th percentile ("high parental depressive symptoms") of the measure.

Youth Negative Mood Symptoms At visits 2–5, youths completed the negative mood symptom subscale from the Child Depression Inventory (Kovacs 1992; the full CDI was not administered in order to reduce participant burden). These six items assessed the extent to which children experienced aspects of negative mood over the past two weeks, specifically, feeling sad, worrying about bad things, feeling like bad things are their fault, feeling like crying, things bothering them, and having difficulty making up their mind (Cronbach's $\alpha = 0.69$ in current sample). The CDI has been shown to have acceptable reliability and validity, including in children as young as 8-years-old (Smucker et al. 1986). Scores on this measure were centered around the participant's average, such that higher scores indicate elevations in negative mood for that visit.

Gene Expression At visits two through five, 2.5 mL of whole blood was drawn into PAXgene Blood RNA tubes through antecubital venipuncture to measure the expression of messenger RNA (mRNA) for the α isoform of the Glucocorticoid Receptor (GR) and for β_2-Adrenergic Receptor (β_2-AR). Total RNA was extracted using PAXgene Blood RNA Kits (Pre- Analytix, Hombrechtikon, Switzerland) and real-time reverse transcription-polymerase chain reaction was executed using commercially-available one-step assays (TaqMan Gene Expression Assay developed in partnership with Applied Biosystems and based on RefSeq NM_000176 for GR-α; TaqMan Gene Expression Assay #Hs00240532 for β_2-AR; Applied Biosystems). Values of each target mRNA were adjusted for expression of a housekeeping gene, 18S, using the delta CT method. Results are expressed as relative quantities of mRNA on a log 2 base scale, where higher values indicate greater expression of the target mRNA and each unit difference in relative quantity indicates a twofold difference in expression.

Covariates To account for the possibility that differences in demographic characteristics might relate to differential gene expression patterns, child age, race (dummy coded for racial minority status), and gender, along with parent gender and family income were assessed at baseline and included as dyad-level covariates (level 2).

To assess whether any observed associations might be accounted for by disease-related factors or by the composition of the circulating leukocyte pool, asthma severity, use of asthma medications in the past week, and leukocyte populations were also assessed. Here, asthma severity was included as a covariate at level 2 and was calculated based on guidelines from the National Asthma Education and Prevention Program/Expert Panel Report 2, in which ratings from 1 to 4 are determined based on medication use and asthma symptom frequency, where 1 = mild intermittent asthma and 4 = severe persistent asthma (Bacharier et al. 2004). To index medication use, at visits 2–5, children reported on the number of days in the preceding 2 weeks that they took an inhaled corticosteroid medication and the number of days in the preceding 2 weeks that they took a beta-agonist medication, which were included as visit-level (level 1) covariates. Lastly, cell subset counts for monocytes, neutrophils, and lymphocytes were obtained from a complete blood count with differential at each visit (ADVIA 70 Hematology System; GMI Inc., Holliston, MA) and included as level 1 covariates.

Analytic Strategy

For our primary analyses, we were interested whether being in a family characterized by higher average parental depressive symptoms would moderate the effects of changes in children's negative mood on youths' expression of GR-α and β_2-AR. To test our hypothesis that children experiencing worse mood would show less gene expression particularly if they had parents with high levels of depressive symptoms, we conducted a series of multilevel models to estimate fixed effects using Hierarchical Linear and Nonlinear Modeling (HLM) software (Version 6.08; Raudenbush et al. 2004). At Level-1, representing the within-person portion of the model, each target gene was separately modeled by youth negative mood (person-centered), along with the covariates of days of inhaled corticosteroid medication use in past week (person-centered), days of beta agonist medication use in past week (person-centered), lymphocyte count (person-centered), monocyte count (person-centered), neutrophil count (person-centered) and a random error term. At Level 2, representing the between-person portion of the model, parents' average depressive symptom score (grand-centered), along with covariates of child gender, child age (grand-centered), child minority racial status, parent gender, family income category (grand-centered), and asthma severity rating (grand-centered) were used to model the intercept and slopes of Level 1 predictors. A

random effect term was included in the Level 2 model of the intercept. A portion of one model is presented below.

$$GR-\alpha_{ij} = \beta_{0j} + \beta_{1j}\left(Youth \quad Negative\,Mood_{ij}\right)$$
$$+ \beta_{2j}\left(Days \quad of \quad Inhaled\,Corticosteriods_{ij}\right)$$
$$+ \beta_{3j}\left(Days \quad of \quad Beta\,Agnoists_{ij}\right)$$
$$+ \beta_{4j}\left(Lymphocyte\,Count_{ij}\right)$$
$$+ \beta_{5j}\left(Monocyte\,Count_{ij}\right)$$
$$+ \beta_{6j}\left(Lymphocyte\,Count_{ij}\right) + r_{ij}$$

$$\beta_{0j} = \gamma_{00} + \gamma_{01}\left(Parent \quad Depression\right)$$
$$+ \gamma_{02}\left(Minority\,Status_j\right) + \gamma_{03}\left(Youth\,Gender_j\right)$$
$$+ \gamma_{04}\left(Parent\,Gender_j\right) + \gamma_{05}\left(Youth\,Age_j\right)$$
$$+ \gamma_{06}\left(Family\,Income_j\right) + \gamma_{07}\left(Asthma \quad Severity\right)$$
$$+ u_{0j}$$

β_{1j} thru β_{6j} modeled similar to above

The main coefficient of interest was the cross-level interaction between parent depression and youth negative mood. A significant interaction term would indicate that parental depression moderated the strength of the association between children's negative mood on the target gene's expression. If a significant interaction term emerged, simple slopes analyses were conducted following techniques developed by Preacher et al. (2006), examining the slope of youth negative mood on gene expression at the 25th percentile (i.e., low parental depression), and 75th percentiles (i.e., high parental depression) of the sample distribution of parental depressive symptoms. Given previous work positing that GR may play a causal role in youth depressive symptoms, models were re-run to examine reverse-directionality in which youths' negative mood was predicted from changes in GR or β_2-AR expression, alone and in interaction with average parental depressive symptoms, adjusting for child age, parent and child gender, family income, minority status, and asthma severity.

Results

Descriptive statistics of study characteristics are presented in Table 1. Results of the multilevel model predicting GR-α expression revealed that, although there was not a main effect of parental depression nor a significant main effect of youth negative mood symptoms, there was a significant cross-level interaction between parent depression and youth mood symptoms wherein the association between visits with higher youth

Table 1 Descriptive characteristics of the sample

Variable	Mean	SD
Youth Age (baseline)	12.61	2.63
Youth Gender	67% Female	
Race/Ethnicity	67% White	
Parent Gender	83% Female	
Income Category (range 2–9)	5.42	1.89
Parental Depressive Symptoms (range 0–33)	9.94	7.30
Youth Negative Mood Symptoms (range 0–8)	1.37	1.50
Days of Inhaled Corticosteroid (past 2 weeks)	5.58	6.23
Days of Beta-Agonist Medication (past 2 weeks)	4.30	5.61
Neutrophils ($\times 10^9$ cells per L)	3.61	1.65
Lymphocytes ($\times 10^9$ cells per L)	2.44	0.77
Monocytes ($\times 10^9$ cells per L)	0.49	0.18
GR-α (relative expression; $\log_2$)	5.21	1.37
β_2-AR (relative expression; $\log_2$)	5.71	2.91

negative mood symptoms and reduced gene expression became increasingly negative at higher levels of parental depressive symptomatology, as displayed in Fig. 1 and Table 2. Tests of simple slopes revealed that the association between negative mood and GR-α expression was marginally significant for youths in families with high parental depression ($b = -0.44$, $SE = 0.24$ $p = 0.078$) but was not significant for dyads with low parental depression ($b = -0.09$, $SE = 0.22$, $p = 0.675$).

The results of the multilevel model predicting β_2-AR revealed a marginally significant main effect of youth mood, which was qualified by a significant interaction between parental depression and child negative mood, wherein children who experienced more negative mood in the context of greater parental depression expressed less β_2-AR (see Fig. 2). As before, main effects of parental depressive symptoms were not significant. Tests of simple slopes showed that the association between mood and gene expression was not significant for dyads with low parental depressive symptoms ($b = -0.18$, $SE = 0.55$, $p = 0.741$), but were significant for children whose parent has high levels of depressive symptoms ($b = -1.76$, $SE = 0.67$, $p = 0.015$).

Post-hoc analyses did not find support for reverse directionality for gene expression predicting youth negative mood. Changes in GR were not related to youth negative mood alone ($b = -0.07$, $SE = 0.09$ $p = 0.453$) nor in interaction with average parental depressive symptoms ($b = -0.01$, $SE = 0.01$, $p = 0.455$). Similarly, there was not a main effect of β_2-AR predicting youth negative mood symptoms ($b = 0.01$, $SE = 0.05$ $p = 0.774$) nor an interaction between β_2-AR and average parental depressive symptoms ($b = -0.01$, $SE = 0.01$, $p = 0.303$).

Taken together, these analyses suggest that parental depressive symptoms potentiate the effect of increases in youth's negative mood on asthma-relevant gene expression, and these effects occur above and beyond contributions of leukocyte subtypes, medication use, demographic characteristics, and asthma severity.

Fig. 1 Interaction between
Average Parental Depressive
Symptoms and Within-Person
Changes in Youth Negative Mood
Predicting Expression of
Glucocorticoid Receptor in
Youths. Note. Low parental
depressive sx = 25th percentile of
sample distribution; high parental
depressive sx = 75th percentile of
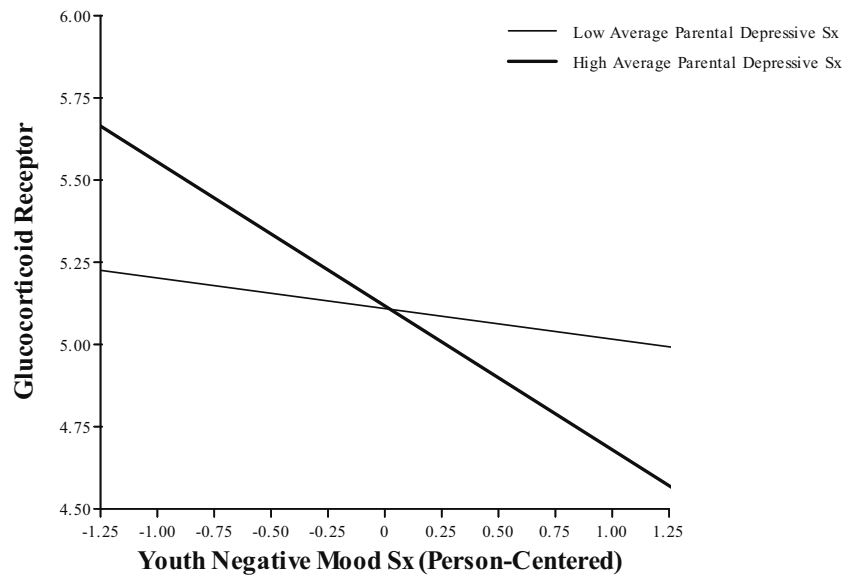sample distribution. GR values
are expressed on a log$_2$ scale

Table 2 Abbreviated results
displaying main effects and cross-
level interaction between youth
negative mood and parental
depressive symptoms

	Coefficient	Standards Error	P-value
Model of GR-α			
Intercept	5.11	0.18	<0.001
Parent Depressive Symptom Average	0.00	0.02	0.951
Asthma Severity	−.12	0.15	0.411
Youth Age	0.07	0.05	0.149
Youth Gender	0.10	0.28	0.717
Parent Gender	−0.56	0.33	0.094
Racial Minority Status	−0.31	0.27	0.257
Income Category	−0.01	0.07	0.876
Child Negative Mood Symptoms	−0.31	0.22	0.173
Parent Depressive Symptom Average	−0.04	0.02	0.034
Days of Inhaled Corticosteroid (past 2 weeks)	−0.03	0.09	0.709
Days of Beta-Adrenergic-Agonist (past 2 weeks)	0.13	0.08	0.098
Neutrophils	0.46	0.21	0.032
Lymphocytes	0.35	0.48	0.462
Monocytes	−0.09	2.07	0.964
Model of β₂-AR			
Intercept	5.56	0.38	<0.001
Parent Depressive Symptom Average	0.00	0.04	0.913
Asthma Severity	−0.18	0.34	0.599
Youth Age	0.21	0.11	0.063
Youth Gender	−0.26	0.67	0.697
Parent Gender	−0.52	0.69	0.453
Racial Minority Status	−0.75	0.61	0.225
Income Category	−0.06	0.17	0.740
Child Negative Mood Symptoms	−1.17	0.59	0.058
Parent Depressive Symptom Average	−0.21	0.06	0.003
Days of Inhaled Corticosteroid (past 2 weeks)	0.32	0.29	0.277
Days of Beta-Adrenergic-Agonist (past 2 weeks)	0.29	0.26	0.277
Neutrophils	1.92	0.63	0.005
Lymphocytes	1.83	1.54	0.246
Monocytes	1.00	5.84	0.866

Right-justified text indicates intercepts of level-1 variables and indented text indicates cross-level interactions with level-2 variables. To conserve space, only main effects and the focal cross-level interaction between youth negative mood symptoms and average parental depressive symptoms are displayed. However, all level-2 variables were included as predictors of all level-1 slopes in the full models. Level-1 variables are person-centered, whereas level-2 variables are grand-centered. Gender was coded such that the majority gender was the referent group; youth gender was coded with 0 = male and 1 = female, whereas parent gender was coded with 0 = female and 1 = male. Racial minority status was coded where 0 = majority and 1 = minority

Fig. 2 Interaction between Average Parental Depressive Symptoms and Within-Person Changes in Youth Negative Mood Predicting Expression of β_2-Adrenergic Receptor in Youths. Note. Low parental depressive sx = 25th percentile of sample distribution; high parental depressive sx = 75th percentile of sample distribution. β_2-AR values are expressed on a $\log_2$ scale
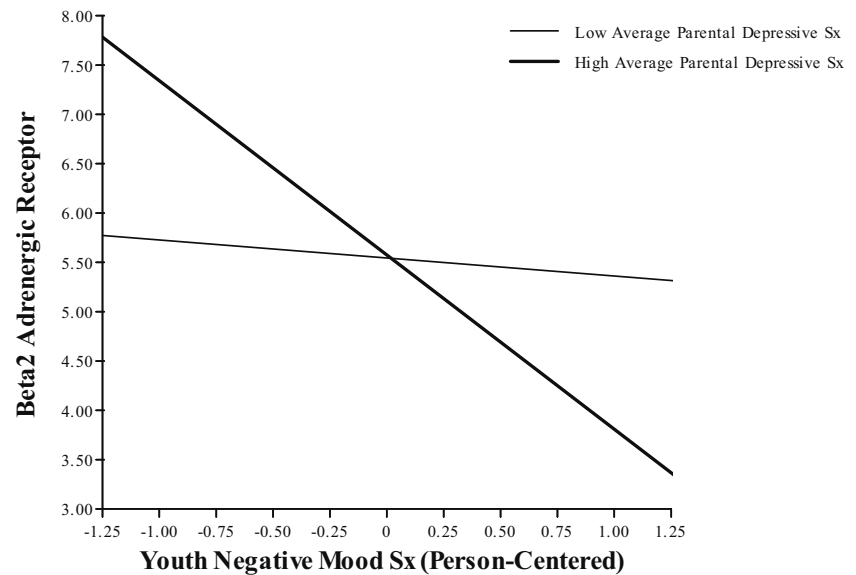
Discussion

The current study demonstrated an interactive effect of parent and youth depressive symptoms on children's asthma-relevant gene expression over a two-year period, suggesting that these mental health processes may affect inflammatory signaling in pediatric asthma. Specifically, it found that the effect of youths' negative mood symptoms on their expression of GR and β_2-AR genes was potentiated by their parents' level of depressive symptoms, such that during periods when children reported more negative mood symptoms than their average, they expressed significantly less GR-α and β_2-AR genes, but this was only true for children who came from families characterized by higher average levels of parental depressive symptoms. Moreover, these associations emerged above and beyond contributions of sociodemographic factors, medication use, asthma severity, and the distribution of leukocyte populations. Together, the present research identifies a biologically-plausible way that psychopathology symptoms in children and their families may lead to worse health processes for children.

That only the combination of youth negative mood in the context of greater average parental depressive symptoms related to less gene expression is consistent with prior research findings that reduced expression of GR and β_2-AR genes in children with asthma was strongest for those experiencing both acute and chronic stress, but that acute stressors alone did not predict gene expression (Miller and Chen 2006). It is also in keeping with robust bodies of work documenting the role of parents in modifying youths' psychopathology symptoms as well as physical health. For example, parental psychopathology symptoms have been shown to potentiate effects of exposure to community violence on youths' PTSD symptoms (Self-Brown et al. 2006). High levels of chronic family stress have been

shown to similarly intensify associations between acute stress and children's mitogen-stimulated production of asthma-relevant cytokines IL-4, IL-5, and IFN-γ (Marin et al. 2009).

These are several explanations for how these associations may develop. One possibility is that parental depression interferes with a mother's or father's ability to act supportively or buffer their children when they experience their own depressive symptoms. Likewise, when children experience more depressive symptoms, they may become less able to cope with the hostility or negative affect that accompanies their parent's depression. These problems may then lead to greater psychological distress on the part of the child, impacting the functioning of their sympathetic nervous system and hypothalamic-pituitary-adrenal axis and resulting in changes in epinephrine and cortisol secretion, for example (Gotlib et al. 2008; Miller et al. 2007), in turn altering inflammatory signaling processes (Miller et al. 2002) and disease dynamics (Miller et al. 2009). An alternative explanation is that associations may be due to lower adherence with medications. However, if this were the case, our associations would not be expected to emerge when statistically accounting for youths' exposure to corticosteroid and beta agonist medications. It remains possible, though, that children and parents with depressive symptoms may be less effective in *how* they administer medication—for example, not taking rescue inhalers until symptoms become extreme or not spacing maintenance medications appropriately. A final explanation for the patterns of results is that congruence between mood symptoms in parents and children may indicate shared genetic liability for depression, such as carrying the short allele for the promotor region of the serotonin transporter gene (5-HTTLPR). Previous work has suggested that carriers of the short allele are more biologically sensitive to environmental input in ways that can relate to depression (Caspi et al. 2010; Vrshek-Schallhorn et al. 2013). In this way, children may show

greater changes in mood symptoms over time as they react to varying situations. It is possible that this sensitivity then contributes to changes in gene expression relevant to physical health.

The current work adds to existing literature on mental and physical health comorbidity by finding evidence of molecular signaling patterns that link youth and family mental health to biological processes relevant to pediatric asthma. In doing so, it identifies a novel aspect of the biological cascades that may contribute to why children with depressive symptoms have worse asthma-related problems, such as more frequent hospital visits (Ahmedani et al. 2013) and increased mortality due to asthma (Strunk et al. 1985). Furthermore, these findings may have important clinical implications. First, our results suggest that it may be important to assess *both* parent and child mental health symptoms during routine pediatric asthma care, rather than focusing exclusively on the mental health of the presenting patient. Second, our work reaffirms the value of considering parenting- and mental health-focused interventions for the treatment of physical health problems. For example, a recent randomized-control trial suggested that family therapy can be effective in reducing pediatric asthma symptoms (Ng et al. 2008). Moreover, by improving both mental and physical health, such interventions may be especially cost-effective, and thus more easily justifiable when clinical and monetary resources are limited.

There are several important limitations to acknowledge in the present study. For example, parental depressive symptoms and youth negative mood symptoms were measured using self-report questionnaires rather than diagnostic interviews or clinician-administered measures. Similarly, there was a somewhat low internal consistency for the measure of child negative mood. Although the self-report measures are well-validated, future work would benefit from more rigorous assessment of psychopathology symptoms. Another limitation is that this set of analyses did not focus on how the observed associations related to history of asthma, or other elements of asthma management, such as exposure to allergens. In addition, the current study was observational, making it difficult to determine causality. For example, it is possible that children's asthma or negative mood may contribute to parent's depressive symptoms. We also were unable to differentiate environmental versus genetic vulnerability. Another limitation is that the study was made up of largely mothers and White families, which may limit generalizability. In addition, children were not able to participate in the current study if they were on a medication for depression or other psychiatric disorder, which likely restricted range. It is also important to acknowledge that while effects may be statistically significant, it is unclear whether they are clinically significant. Lastly, the present study focused on two target genes that have been implicated in asthma and asthma-management. However, this approach of limiting focus to the expression of two genes has the potential to overlook other associations that might emerge using genome-wide analysis.

The current research also raises questions for future work. For example, additional research should probe which aspects of parental or youth depressive symptoms most contribute to the pattern of associations. For instance, is it a lack of support from parents, greater parent-child conflict, children's impaired emotion regulation, or greater family unpredictability that is most relevant to gene expression? Moreover, given that maternal and paternal depression appear to have different associations with youth mental health (Pilowsky et al. 2014), it will also be important for future work to include both parents to explore gender differences for these patterns of results. Additionally, it will be critical to test these processes within other pediatric disease models to examine whether parent and child depressive symptoms relate to the expression of other genes. By identifying common and disease-specific associations, future research can better understand processes associated with depression and depression comorbidity. Finally, given that pediatric diseases, including asthma, occur across periods of tremendous social-emotional and physical change, future research should more closely examine developmental shifts in symptoms and gene expression. Possible dynamics to explore would be whether associations are stronger for younger children and whether associations change once children move away from home. As children age, it is possible that the mental health of other relationship partners, such as close friends or romantic partners, may become more relevant to youths' mental health and gene expression than parental depressive symptoms.

Regardless of these limitations and unanswered questions, the current work is the first to document interactive associations between parent- and youth-depressive symptoms on gene expression in a sample of children with asthma. As such, it identifies novel and biologically-proximal molecular signaling patterns that connect depressive symptoms to pediatric asthma while also highlighting the important role of family environment for biological processes that may operate within depression comorbidity.

Compliance with ethical standards

Conflict of interest　The authors declare that they have no conflict of interest.

Ethical Approval　The authors attest that they have complied with ethical standards, including, but not limited to, oversight by the Institutional Review Board of the University of British Columbia.

Informed Consent　Informed consent/assent was obtained from all participants.

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