HHS Public Access

Author manuscript

Psychol Sci. Author manuscript; available in PMC 2016 February 01.

Published in final edited form as:

Psychol Sci. 2015 February ; 26(2): 111–121. doi:10.1177/0956797614556320.

Targeted rejection predicts decreased anti-inflammatory gene expression and increased symptom severity in youth with asthma

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Abstract

Although responses to stress are sometimes assumed to be similar across different stressors, recent research has demonstrated that certain types of stress, such as targeted rejection, are particularly impactful. To test such associations in a chronic disease model, we examined how non-interpersonal, interpersonal, and targeted rejection life events predicted changes in gene expression and symptom severity in 121 youth with asthma who were assessed every 6 months for 2 years. Youth who recently experienced targeted rejection had less mRNA for signaling molecules that control airway inflammation and obstruction, specifically the glucocorticoid receptor and β2-adrenergic receptor. These associations were specific to targeted rejection and stronger for higher-status youth. Higher-status youth exposed to targeted rejection (but not other types of stress) also exhibited more asthma symptoms. These data demonstrate stressor-specific associations with molecular signaling pathways and asthma disease severity, and suggests threats to the social self may be particularly deleterious.

Keywords

social rejection; social status; asthma; gene expression; health

Stress research has been heavily shaped by the view that biological responses are uniform across different stressors (Selye, 1973). Although influential, this perspective has been criticized by theorists who argue that, to be adaptive, stress responses must be sensitive to

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Author Contributions: EC and GEM designed the original study from which the data for this manuscript were obtained; MLMM, GMS, and GEM developed the idea for this manuscript; MLMM conducted the data analyses; all authors were involved in writing the paper; and all authors read and approved the final manuscript.

the heterogenous demands of different situations (Kemeny, 2003; Mason, 1971; Weiner, 1992). Supporting this latter formulation, some evidence demonstrates that different stressful situations have distinct physiological signatures (Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009; Dickerson & Kemeny, 2004). But these findings derive almost exclusively from brief laboratory studies and, consequently, at least two important questions remain unanswered. Namely, does stressor specificity extend to stressful life events that people experience in the real world and, if so, are there corresponding implications for health?

To address these questions, we examined how a specific stressor, targeted rejection, relates to the health of children with asthma. Targeted rejection is a unique type of stressor insofar as it entails the intentional rejection of a person by another person or group of people (Slavich, Thornton, Torres, Monroe, & Gotlib, 2009). Examples of targeted rejection include having a romantic partner end one's relationship or being fired from a job. Compared to similar events with different social-psychological characteristics – for example, choosing to end a relationship or being laid off from work along with other employees – targeted rejection elicits depression more rapidly and provokes larger changes in hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system (SNS), and inflammatory activity (Slavich & Irwin, 2014; Slavich, O'Donovan, Epel, & Kemeny, 2010). Targeted rejection's links with these systems may be a vestige of our evolutionary history. Humans have a fundamental need to belong (Baumeister & Leary, 1995), and social exclusion elicits biological responses (Dickerson & Kemeny, 2004). Theorists believe these responses prepare the body to manage threats, including injuries and infections, that accompanied exclusion in socially hostile ancestral environments (Dickerson, Gruenewald, & Kemeny, 2011; Irwin & Cole, 2011; Slavich et al., 2010). Although adaptive in the ancestral context, and perhaps still today, these responses may compromise health when evoked repeatedly. For example, persistent inflammation is known to contribute to the development of many chronic diseases (Nathan & Ding, 2010).

Building on these observations, we examined how targeted rejection relates to health in a multi-wave study of youth with asthma. We also compared the impact of targeted rejection to other stressors of similar severity. Indeed, targeted rejection may be especially impactful during later childhood and adolescence. During these periods, social activities become increasingly important sources of identity development (Hansen, Larson, & Dworkin, 2003), and youth report increased affiliation with peers and concerns about their position within social networks (Larson & Richards, 1991; Spear, 2000). As a pediatric health condition, asthma is ideal to study because it is well understood (Wright, 2011), a major cause of disability and impairment, and poses a significant economic burden (Akinbami, Moorman, Garbe, & Sondik, 2009). The pathogenesis of asthma involves inflammation and airway obstruction that results in coughing, wheezing, shortness of breath, and chest tightness. Among the molecules that regulate airway inflammation and obstruction are the hormones cortisol, epinephrine, and norepinephrine, which act through binding to the glucocorticoid receptor (GR) and the β2-adrenergic receptor (β2-AR). Accordingly, we hypothesized that targeted rejection would be associated with decreased expression of both GR and β2-AR, and a corresponding increase in the severity of asthma symptoms.

To examine the specificity of these associations, we compared targeted rejection's impact with a general category of interpersonal stressors. This enabled us to better isolate the unique effects of rejection. To evaluate the generality theories that have pervaded stress research (Selye, 1973), we also created a broader category of non-interpersonal stressors. Based on the aforementioned research, we hypothesized that targeted rejection would relate to biological and clinical outcomes in asthma more strongly than similarly severe, but contextually different interpersonal or non-interpersonal life events.

Each individual possesses unique resources that moderate their biological responses to stressful encounters and subsequent health outcomes (Weiner, 1992). Consequently, we also tested for possible moderation. One factor that is likely to moderate associations between targeted rejection and health is subjective social status, or where youth believe they are situated within their peer hierarchy (Goodman et al., 2001). Although in the aggregate, higher-status individuals generally exhibit better health (Adler et al., 1994; Goodman, 1999), mounting evidence suggests that stature-threatening events take a disproportionately greater toll on these individuals. For example, one study exposed young adults to negative social evaluation and found significantly larger cortisol responses in high- versus low-status participants (Gruenewald, Kemeny, & Aziz, 2006). We recently replicated and extended these findings in adolescents, focusing on how social status moderates the effects of targeted rejection. Although, overall, adolescents who experienced targeted rejection showed increased expression of genes involved in immune system regulation, these associations were moderated by social status, with adolescents higher in subjective social status exhibiting the largest targeted rejection-related changes in gene expression (Murphy, Slavich, Rohleder, & Miller, 2013). These findings are consistent with studies of male non-human primates, where social instability evokes larger biological stress responses and more coronary artery disease in higher- versus lower-ranking animals (Manuck, Marsland, Kaplan, & Williams, 1995). These exaggerated responses are thought to reflect the disproportionate cost of the encounter for high-status animals, in terms of access to resources and safety within the peer group. High-status adolescents who experience targeted rejection may incur similar costs; in addition, rejection may violate their sense of justice and undermine their self-esteem more so than for lower-status individuals (Ramos, Correia, & Alves, 2014). Drawing on this work, we hypothesized that targeted rejection's association with asthma outcomes would be moderated by subjective social status. Specifically, we expected individuals who viewed themselves as being higher in social status to have the steepest declines in GR and β2-AR, and the greatest increases in asthma symptom severity.

Method

Participants and Procedures

Data were collected as part of a longitudinal study of youth with asthma. Participants were recruited using newspaper advertisements and flyers posted at schools and community centers, and from asthma clinics, in and around Vancouver, British Columbia. To be eligible, individuals were required to be (a) 9 – 18 years old, (b) fluent in English, (c) have received a diagnosis of asthma from a physician, (d) free of acute respiratory illnesses at the time of a visit, (e) medically stable, as defined by not having a course of oral steroids for the

past 2 weeks, and (f) have no chronic psychiatric or physical illnesses other than asthma. A total of 121 individuals met these criteria and were enrolled in the study (83% had allergic asthma). The project was approved by the University of British Columbia Research Ethics Board. A parent provided written consent, and youth provided assent (see Table 1 for overview of sample information; see Table S1 for complete descriptive information for the sample within each visit; see Table S2 for a correlation matrix of baseline study variables).

Participants came into the laboratory every 6 months for 2 years, providing up to 5 waves of data. At each wave, they completed questionnaires, were administered interviews, and underwent a blood draw. Following each visit, individuals reported on asthma symptoms daily for two weeks. Overall, 104 participants (86%) completed at least 3 waves, and the majority of participants (n = 86, 71%) completed all 5 waves. Analyses were based on all available data.

Life Stress and Targeted Rejection

We used the Life Stress Interview (LSI; Hammen, 1991) to identify the different types of life events that each participant experienced over the study period. Trained interviewers probed participants to understand the precise nature and contextual features of the stressors they experienced during the six months prior to each study visit. The interviewer presented this information to a team of at least three independent raters, being careful not to disclose details that could have biased the ratings, such as participants' emotional responses to the events. The rating team then made consensus judgments regarding severity based on how much the event would be expected to impact a typical person, given a similar context. Ratings were made in half-point increments on a 1 (*no negative impact*) to 5 (*severe impact*) scale.

Research examining the effects stressful life events have on health has yielded substantial evidence that the impact of such events follows a threshold model, with a distinction being made between "major" and "minor" life events (Monroe & Simons, 1991; Monroe, Slavich, & Georgiades, 2009). Most studies that have employed the LSI to conduct this research have coded events falling at or above the midpoint of the severity rating scale (i.e., 2.5) as being "major" (e.g., Miller & Chen, 2006; Murphy et al., 2013; Slavich, Tartter, Brennan, & Hammen, 2014; Uliaszek et al., 2012; Vrshek-Schallhorn et al., 2013), and we followed this empirical precedent and standard practice in the present study by focusing on life events that were rated 2.5 or higher in severity. These events were subsequently coded as interpersonal or non-interpersonal by author M.L.M.M., with interpersonal events being defined as an event where the main feature involved difficulties with another person or group of people. Finally, interpersonal events were also coded for targeted rejection by author M.L.M.M., following criteria outlined by Slavich et al. (2009). Specifically, an interpersonal event was judged to involve targeted rejection if it (a) happened primarily to the participant, (b) involved the rejection of the participant by another person or group, (c) involved a clear intent to actively reject the participant, (d) directly affected the participant, and (e) resulted in the severing of a social bond between the participant and the other person or group. Seventeen major life events met criteria for targeted rejection. The majority of these targeted rejection major life events (n = 15) were experienced by different participants, and the

maximum number of targeted rejection life events experienced by any participant was two. Of these events, seven involved a romantic breakup, six involved problems with friends or other peers at school, and four involved problems at school, such as expulsion (for examples of these events, see Table S3).

Although working with a larger pool of events would be ideal, targeted rejection appears to be a relatively low base-rate stressor. For this project, we conducted more than 500 interviews, from which we identified 139 major life events and (from those events) 17 targeted rejection major life events. Comparatively, in a previous paper from our group on targeted rejection, using a separate, independent sample of adolescents, we conducted more than 750 interviews, from which we identified 165 major life events, of which 20 met criteria for targeted rejection (Murphy et al., 2013). These base rates are also consistent with the original paper by Slavich et al. (2009), which examined associations between targeted rejection and onset of depression.

Ultimately, this coding process resulted in three mutually exclusive major life event categories: non-interpersonal, interpersonal, and targeted rejection. To examine the reliability of coding interpersonal events, a randomly selected subset of events (n = 10; 5 events originally coded as interpersonal, 5 events originally coded as non-interpersonal) were independently evaluated by G.M.S., who was blind to both the original ratings and the number of life events coded as either interpersonal or non-interpersonal. This same procedure was followed to examine the reliability of coding targeted rejection events. Agreement between coders was excellent for both categories (κ = 1.00 for interpersonal events; κ = 0.80 for targeted rejection).

Subjective Social Status

Participants' subjective social status within their peer group was assessed at baseline using the MacArthur Scale of Subjective Social Status Youth Version (Goodman et al., 2001). Participants were shown a picture of a ladder with 10 rungs and asked to indicate where they felt they fit on the ladder compared to their peers at school. They were told that higher rungs corresponded to people in their school who were respected, had friends, and did well academically, whereas lower rungs indicated peers who were not respected, did not have friends, and received poor grades.

Asthma Symptoms

Self-reported asthma symptoms were obtained twice daily for two weeks following each study visit. After waking, participants reported how bothered they were by coughing, wheezing, chest tightness or pain, and shortness of breath during the previous night. At bedtime, they reported how bothered they were by these same symptoms during the day. Values ranged from 0 (*none*) to 4 (*really bad*). Symptom reports were averaged across the two weeks.

GR and β2-AR

Antecubital blood was collected at each visit to measure leukocyte mRNA for the α isoform of GR and β2-AR. 2.5ml of whole blood were collected into PAXgene Blood RNA tubes

and total RNA was extracted using PAXgene Blood RNA Kits (Pre-Analytix, Hombrechtikon, Switzerland). RT-PCRs were carried out using commercially available one-step assays from Applied Biosystems (TaqMan Gene Expression Assay developed in partnership with Applied Biosystems and based on RefSeq NM_000176 for GRα; TaqMan Gene Expression Assay #Hs00240532 for β2-AR; Applied Biosystems) on an Applied Biosystems Sequence Detection System (Applied Biosystems, Foster City, CA). Using the delta CT method, values of each target mRNA were adjusted for expression of the housekeeping gene, 18S, which was measured in parallel (TaqMan Gene Expression Assay #Hs99999901; Applied Biosystems). Results were expressed as relative quantities of mRNA, with higher values indicating greater expression of the target mRNA relative to the sample range. Each unit difference in relative quantity indicates a twofold difference in expression.

Why are GR and β2-AR of specific interest? As described earlier, asthma is characterized by inflammation and obstruction of the airways, brought about by exaggerated physiologic responses to allergens, infections, and other stimuli. Normally, the body uses hormones it produces endogenously to help keep these processes in check. Cortisol regulates inflammation by acting on GRs located inside cells of the immune system, and the catecholamines epinephrine and norepinephrine regulates the tone of the airways by acting on β2-AR located on smooth muscle cells in the lungs. Dyregulation of these signaling pathways is a central pathologic feature of many cases of asthma, which is why physicians often treat asthma with medications that target these receptors. Corticosteroids are the principle controller medication for many asthma patients, as they help down-regulate inflammation by acting on GRs inside cells of the immune system. Beta-agonists, in turn, serve as rescue medications for many asthma patients who are in the midst of an acute attack. These agents widen the airways and ease breathing by binding to β2-AR on airway smooth muscle cells; they also act on immune cells in ways that reduce airway inflammation.

Alternative Explanations

Exposure to major life events varies across demographic groups and so do asthma outcomes. To evaluate potential demographic confounds, we collected data on age, gender, and ethnicity (i.e., Caucasian or other), and included these variables as covariates in analyses. Major life events may also lead to weight gain, or vice-versa, and obesity affects inflammatory processes and asthma outcomes (Mokdad et al., 2003; Pirkola et al., 2010). To evaluate the possibility of confounding by obesity, we measured body mass index (BMI) at each visit. BMI was highly stable across the study (*ICC* = 0.90), so we used average BMI values as covariates. Additionally, recognizing that life events may alter asthma medication use, and that these medications directly modulate physiological processes related to asthma, we assessed the number of days that participants reported using the two most common categories of asthma medications (i.e., inhaled corticosteroids or β-agonists) during the two weeks prior to each visit. This enabled us to test whether life events related to health outcomes above and beyond medication use[1]. Finally, to evaluate whether links between life

[1]Models analyzed without adjusting for medication use yielded the same pattern of findings.

event exposure and mRNA levels were confounded by variations in the composition of the circulating leukocyte pool, we covaried cell subset numbers obtained from a complete blood count with differential at each visit (ADVIA 70 Hematology System, GMI Inc., Holiston, MA).

Analytic Approach

Analyses were based on all available life stress data, gene expression data – specifically levels of GR and B2-AR mRNA – and asthma symptoms levels assessed repeatedly up to 5 times over a two-year period. We also assessed participants' levels of subjective social status at baseline. Our predictors of interest included participants' baseline subjective social status and whether they experienced a recent non-interpersonal, interpersonal, or targeted rejection major life event during the six-months prior to each of the five study visits. The main outcomes of interest were within-person changes in levels of mRNA for GR and β2-AR, which were measured at each study visit, as well as within-person changes in participants' asthma symptoms, which were assessed daily over a two-week period following each study visit and then averaged over that period to form an index of asthma symptom severity at each time point.

To test our hypotheses, we evaluated a series of multilevel models using the software HLM 6.08 (Raudenbush, Bryk, & Congdon, 2004). For the within-person portion of the models (Level 1), in separate equations[2], the outcome variable of interest was predicted by within-person changes (i.e., the variables were person-centered) in either the occurrence of a (a) non-interpersonal major life event, (b) interpersonal major life event, or (c) targeted rejection major life event. Time since baseline (in months), inhaled corticoid steroid use, β-agonist use, and a random intercept term were also included at Level 1 in all models. For the between participant portion of the models (Level 2), the Level 1 slopes and intercept were predicted by individual differences in subjective social status, as well as age, gender, ethnicity, and BMI. Subjective social status, age, and BMI were mean centered. The two key coefficients of interest for these analyses were (a) the Level 2 intercept for the different stress variables, representing the extent to which the occurrence of each type of life event was associated with within person changes in the outcome of interest, and (b) the cross-level interaction between subjective social status and stress occurrence. A significant interaction indicated that subjective social status moderated the strength of the association between within-person changes in the occurrence of a major life event and the outcome of interest. Simple slopes analyses for the cross-level interactions at the mean (6.60) and 25th and 75th percentile (5.99 and 7.99, respectively) of the sample distribution of subjective social status were calculated using techniques outlined by Preacher, Curran, and Bauer (2006). Finally, multilevel model effect sizes, *d*, were obtained using methods described by Tymms (2004). Table S4 contains the regression coefficients, along with their corresponding standard errors and *p*-values, for each variable in the models.

[2]Although the most ideal strategy for testing whether targeted rejection is uniquely related to asthma-related outcomes involves including all types of life events in a single model, this was not possible in the present study, given the additional degrees of freedom (which translates into two more waves of data collection) that would be required.

We used bar graphs to depict associations between exposure to a recent targeted rejection major life event at each study visit and the outcomes of interest for participants reporting different levels of subjective social status. The bars in these graphs do not represent the means for distinct groups of data, but rather the predicted values of the dependent variables, plotted from the multilevel model regression coefficients. As such, the predicted values shown in the bar graphs are based on all available data (i.e., the full sample for which data is available for visits where no event occurred, and the full sample of 17 targeted rejection major life events for visits when such an event occurred).

Results

Comparing the Severity of Different Types of Major Life Events

It is possible that stronger associations between targeted rejection life events and asthma related outcomes could simply be due to the fact that targeted rejection events are more severe in nature than interpersonal and non-interpersonal life events. To address this possibility, we compared the objective team-rated severity ratings of non-interpersonal, interpersonal, and targeted rejection major life events. We found no evidence of differences in the severity between these categories of events (non-interpersonal: $M = 3.0$, $SD = .52$; interpersonal: $M = 3.0$, $SD = .53$; targeted rejection: $M = 3.1$, $SD = .57$), $F(2,138) = .47$, $p = .63$. Consequently, any differences detected in the associations of these three different types of life events with asthma-related outcomes are likely due to differences in their underlying social-psychological characteristics as opposed to differences in their basic stressfulness or severity.

Major Life Events, Subjective Social Status, and Glucocorticoid Receptor (GR) mRNA

We found significant associations between within person changes in the occurrence of targeted rejection and the relative expression of GR mRNA (see Figure 1). As predicted, at visits when participants had experienced targeted rejection within the past six months, they exhibited significantly less mRNA for GR compared to visits when no targeted rejection had occurred ($b = -1.62$, $SE = .65$, $p = .01$, $d = -.42$). Additionally, individual differences in subjective social status moderated the strength of the association between targeted rejection and GR mRNA ($b = -.46$, $SE = .19$, $p = .02$). Simple slopes analyses indicated that individuals higher in status showed a sharper decline in mRNA for GR following a recent targeted rejection major life event ($b = -2.26$, $SE = .78$, $p = .004$, $d = -.59$) than individuals lower in status ($b = -1.34$, $SE = .62$, $p = .03$, $d = -.35$). (Note that the association between targeted rejection and GR mRNA at the mean of subjective social status is identical to the results describing the associations between within person changes in the occurrence of targeted rejection and the relative expression of GR mRNA presented first in this paragraph, as subjective social status was included in all models as a centered variable with a mean of 0.) This pattern remained after adjusting for variations in the distribution of circulating leukocytes in a separate model ($b = -1.77$, $SE = .65$, $p = .008$ for the association; $b = -.51$, $SE = .19$, $p = .007$ for the interaction).

We conducted parallel analyses to examine whether these patterns held for other similarly severe stressors. However, we did not find significant associations between within-person

changes in the presence of non-interpersonal (b = .82, SE = .44, p = .07, d = .36) or interpersonal major life events (b = .21, SE = .53, p = .70, d = .01) and expression of GR mRNA. Furthermore, individual differences in subjective social status did not moderate associations between non-interpersonal (b = −.15, SE = .14, p = .31) or interpersonal major life events (b = −.15, SE = .11, p = .15) and GR mRNA. These findings are consistent with the notion that associations between major life events and GR mRNA are specific to targeted rejection.

Major Life Events, Subjective Social Status, and β2-Adrenergic Receptor (β2-AR) mRNA

In addition to GR mRNA, we found a significant association between within person changes in the occurrence of targeted rejection and the relative expression of β2-AR mRNA (see Figure 2). As predicted, at visits when participants had experienced targeted rejection within the past six months, they exhibited significantly less mRNA for β2-AR compared to visits when no targeted rejection had occurred (b = −3.20, SE = 1.14, p = .006, d = −.51). Furthermore, individual differences in subjective social status moderated the strength of the association between targeted rejection and β2-AR mRNA (b = −.59, SE = .30, p = .05). Simple slopes analyses revealed that individuals higher in subjective social status showed a larger decline in β2-AR mRNA following a recent targeted rejection major life event (b = −4.01, SE = 1.15, p = .001, d = −.64) than individuals lower in status (b = −2.84, SE = 1.19, p = .02, d = −.45). This pattern of findings remained after adjusting for variations in the distribution of circulating leukocytes in a separate model (b = −2.46, SE = 1.26, p = .05 for the association; b = −.55, SE = .30, p = .07 for the interaction).

Do other comparably severe stressful life events show similar associations with changes in β2-AR mRNA? No, we did not find associations between within person changes in the occurrence of non-interpersonal (b = 1.03, SE = .91, p = .20, d = .28) or interpersonal major life events (b = −.16, SE = .96, p = .87, d = −.05) and expression of β2-AR mRNA. Likewise, subjective social status did not moderate the strength of the association between non-interpersonal (b = −.41, SE = .27, p = .13) or interpersonal major life events (b = −.11, SE = .17, p = .52) and β2-AR mRNA expression. These findings are consistent with the notion that associations between major life events and β2-AR mRNA are specific to targeted rejection.

Major Life Events, Subjective Social Status, and Asthma Symptoms

There were no within-person associations between the occurrence of targeted rejection and asthma symptoms, which were measured during the two-week period following each study visit (b = .07, SE = .10, p = .48, d = .11). However, there was a significant cross-level interaction with subjective social status (See Figure 3; b = .18, SE = .04, p < .001). Simple slopes analysis revealed that individuals who were lower in social status did not report targeted rejection-related change in asthma symptoms (b = −.04, SE = .10, p = .72, d = −.05). Consistent with the gene expression findings described above, however, participants higher in social status who experienced a recent targeted rejection major life event did report significantly more asthma symptoms during the two-week period following their study visit (b = .32, SE = .12, p = .008, d = .47).

Consistent with the gene expression findings described above, there were no associations between the occurrence of non-interpersonal or interpersonal major life events and changes in asthma symptoms (non-interpersonal: $b = -.07$, $SE = .11$, $p = .51$, $d = -.19$; interpersonal: $b = .06$, $SE = .07$, $p = .36$, $d = .17$). Likewise, subjective social status did not moderate associations between non-interpersonal or interpersonal major life events and asthma symptoms (non-interpersonal: $b = .06$, $SE = .03$, $p = .08$; interpersonal: $b = -.02$, $SE = .01$, $p = .14$).

Mediation Analyses

Next, we examined whether changes in the relative expression of mRNA for GR and β2-AR mediated the association between targeted rejection and self-reported asthma symptoms. To warrant testing for mediation, in addition to there being associations between targeted rejection and both mRNA and symptoms, changes in mRNA should also predict changes in symptoms. However, we did not find any evidence that changes in symptoms were predicted by within-person changes in mRNA for GR ($b = .01$, $SE = .02$, $p = .76$) or β2-AR ($b = -.01$, $SE = .01$, $p = .41$). When taking subjective social status into account, we found weak evidence that within person changes in mRNA for GR predicted changes in symptoms as a function of subjective social status ($b = .02$, $SE = .01$, $p = .05$), and no evidence that changes in mRNA for β2-AR predicted changes in symptoms as a function of social status ($b = .01$, $SE = .01$, $p = .13$). As such, there was no evidence that changes in the relative expression of mRNA for GR and β2-AR mediated the association between targeted rejection and self-reported asthma symptoms.

Subjective Social Status and Life Event Exposure and Severity

Finally, we examined whether participants exposure to, or the severity of, the major life events that they experienced varied as a function of their subjective social status. To test these effects, we used multilevel modeling to compute the odds of experiencing a major life event during the study as a function of individual differences in subjective social status. We found no evidence for differential exposure to life events by subjective social status for non-interpersonal (OR = .99, $b = -.01$, $SE = .01$, $p = .21$), interpersonal (OR = .99, $b = -.01$, $SE = .01$, $p = .10$), or targeted rejection (OR = 1.00, $b = -.004$, $SE = .01$, $p = .76$) major life events. Next, we examined whether there were any differences in the severity of the major life events that participants experienced as a function of their subjective social status. Again, we found no evidence for differential severity of life events by social status for non-interpersonal ($b = .002$, $SE = .003$, $p = .65$), interpersonal ($b = -.003$, $SE = .003$, $p = .41$), or targeted rejection ($b = .003$, $SE = .004$, $p = .44$) major life events. Taken together, these findings suggest that the effects that we observed on asthma outcomes were not simply due to social status-related differences in participants' exposure to, or the severity of, the major life events that participants of different social stature experienced.

Discussion

In a two-year multi-wave study, we found consistent within-person associations between targeted rejection and the expression of signaling molecules that regulate airway inflammation and obstruction, in addition to asthma symptom severity. These data are

among the first to provide life event-related evidence supporting the specificity hypothesis (Kemeny, 2003; Mason, 1971; Weiner, 1992), which posits that stressors elicit relatively distinct biological responses with corresponding consequences for health. A key feature of targeted rejection is social-evaluative threat (Slavich et al., 2010), which is relatively unique among major life events in terms of its capacity to engage neural pain and threat networks that upregulate HPA axis, SNS, and inflammatory activity (Dickerson & Kemeny, 2004; Kemeny, 2009). As we noted earlier, upregulation of these systems following social rejection or exclusion would have been adaptive in the ancestral context. But our data suggest that for asthma patients in the modern world, targeted rejection may undermine health, whereas other similarly severe stressors do not. In future research, it will be important to replicate these patterns, and further delineate the neural, endocrine, and immune pathways that are engaged by targeted rejection and underlie its effects.

We also found that subjective social status moderated targeted rejection's association with asthma outcomes. Higher-status youth exhibited the greatest declines in gene expression, and the largest increases in symptom severity, following targeted rejection. These findings replicate previous research on social status as a moderator (Gruenewald, Kemeny, & Aziz, 2006; Murphy et al., 2013), and relate this work to a clinical health outcome. Our results also converge with research on non-human primates, which has shown that social instability (Manuck et al., 1995) and stature loss (e.g., Shively & Clarkson, 1994) have more pronounced health consequences for high- versus low-status animals.

This study is not without limitations. First, its design was correlational. Although data were collected prospectively across five waves and several possible confounding factors were statistically adjusted for, definitive causative inferences cannot be made. At the same time, most analyses were based on within-person comparisons, and their results cannot logically be confounded by individual difference factors that typically threaten interpretations in correlational research. Regarding the moderation analyses, social status is unlikely to be distributed at random, and there are several factors that could potentially explain differential responses to targeted rejection in high- versus low-status individuals, including neuroticism, excessive reassurance seeking, narcissism, and rejection sensitivity. Second, although targeted rejection was associated with changes in signaling molecule expression and everyday symptom reports, we did not find evidence for a mediational scenario. This is not surprising given that many regulatory processes occur between gene expression and symptom presentation. Third, although levels of GR and β2-AR mRNA were derived from peripheral leukocytes, asthma exacerbations occur because of perturbations in the cells that make up the airways. Moreover, changes in the peripheral expression of the genes that code for these receptors does not guarantee that such changes also occur in the airways. Additional research that harvests cells from the airways is thus needed to better understand these processes in relevant tissue. Fourth, this study focused only on youth with asthma who reported experiencing major life events. It will be important for future research to examine whether these findings generalize to other, less severe forms of rejection, and whether subjective social status moderates such associations in a similar way. It will also be important to study whether these findings replicate across different chronic illnesses and developmental periods.

Another limitation concerns the relatively small number of targeted rejection events in the sample. There are several reasons why this may be of concern. First, with such a low base rate, power to detect associations is low. Since we did find strong associations, this is not an issue for the current study. Second, fewer life events could lead to less precise estimates. However, this did not seem to be the case, as associations for targeted rejection emerged consistently across both biological and symptom outcomes, which themselves are weakly correlated. Furthermore, the standard errors associated with the multilevel model parameter estimates were not large. We suspect that any problems with precision were offset by the high inter-rater reliability that we achieved using a well-validated, semi-structured interview and strict coding guidelines. Finally, there could be concern that the findings are unstable or spurious. Although this cannot be ruled out, targeted rejection showed consistent associations across multiple outcomes, and such relations were not present for other life events of similar severity. Furthermore, these gene expression findings replicate another study (Murphy et al., 2013) that used an independent sample of adolescents reporting similar rates of targeted rejection. Therefore, although a higher base rate of targeted rejection life events would be ideal, we believe it is unlikely that any study could identify more targeted rejection events without conducting thousands of intensive stress interviews and concomitant assessments of biology, which is extremely costly and likely unfeasible.

Notwithstanding these limitations, this study offers new theoretical insights. Specifically, in a prospective, multi-wave study, we found some of the first real-life evidence to support the stressor-specificity hypothesis in relation to health. These results challenge the uniformity assumption that has dominated theory and research on stress for much of the past century. In addition, the findings highlight the moderating role of subjective social status, extending the observation from animal research that social instability takes a disproportionately greater toll on the health of higher-ranking individuals. More broadly, these findings deepen our knowledge of how social adversity reaches inside the body to influence health (Slavich & Cole, 2013).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Katherine Ehrlich for her helpful feedback during the early stages of this project. We also wish to thank Shreya Agarwal, Nova Hou, Victoria Larsen, and Julia Wolloch for their assistance with preparing data for this manuscript.

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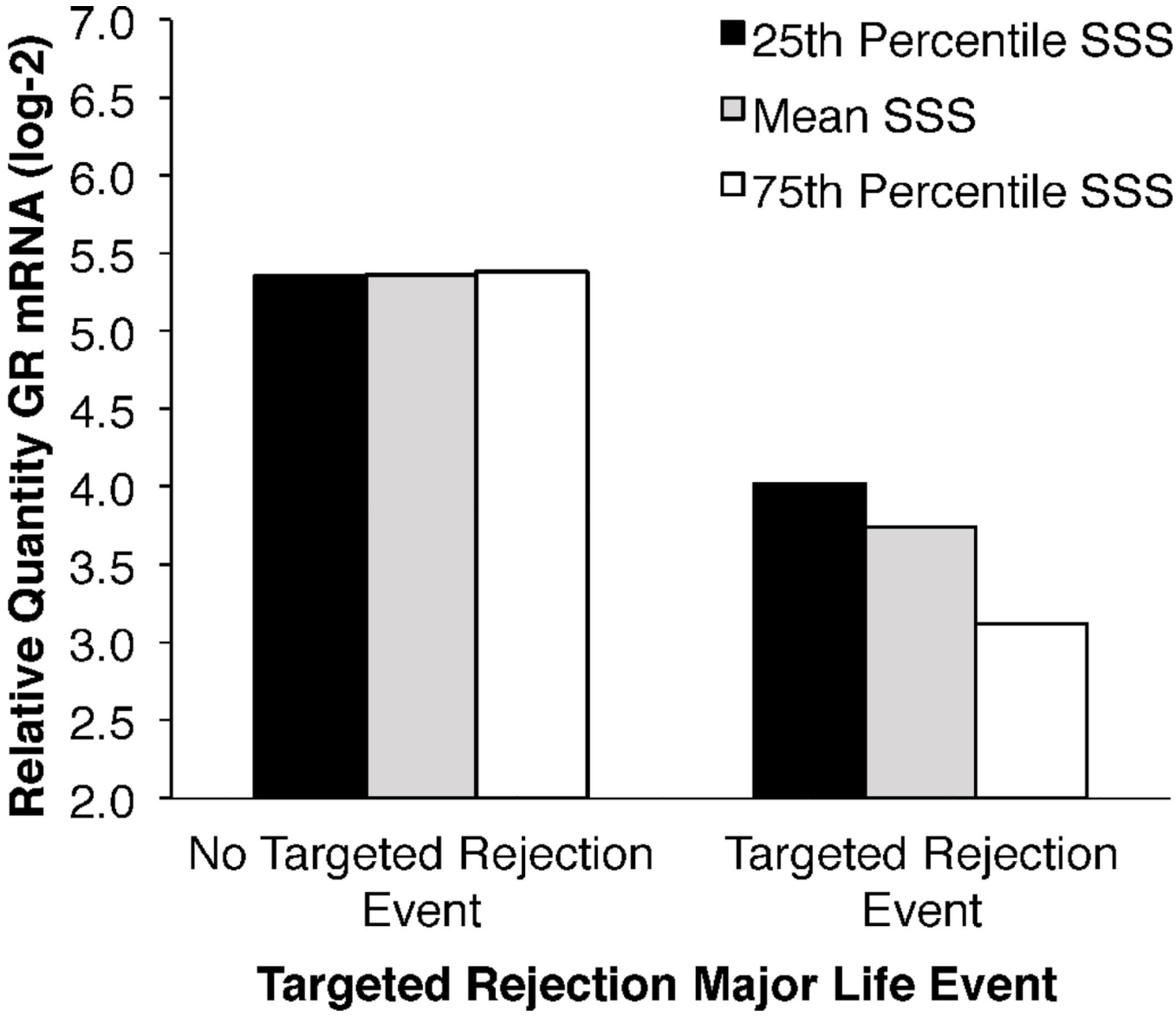

Figure 1.

Regression-based point estimates of the differences in messenger RNA (mRNA) expression for glucocorticoid receptor (GR) following the experience of a recent targeted rejection major life event (n = 17) for participants reporting different levels of subjective social status (SSS). At visits when participants had experienced a recent targeted rejection major life event, they exhibited less mRNA for GR, p = .01, d = −.42. Moreover, this association was moderated by SSS, p = .02, with a simple slopes analysis revealing that youth higher in SSS showed the steepest declines in expression of GR following targeted rejection, p = .004, d = −.59. Individual bars represent the point estimate (not observed means) for GR mRNA based on the multilevel model regression coefficients (N = 121).

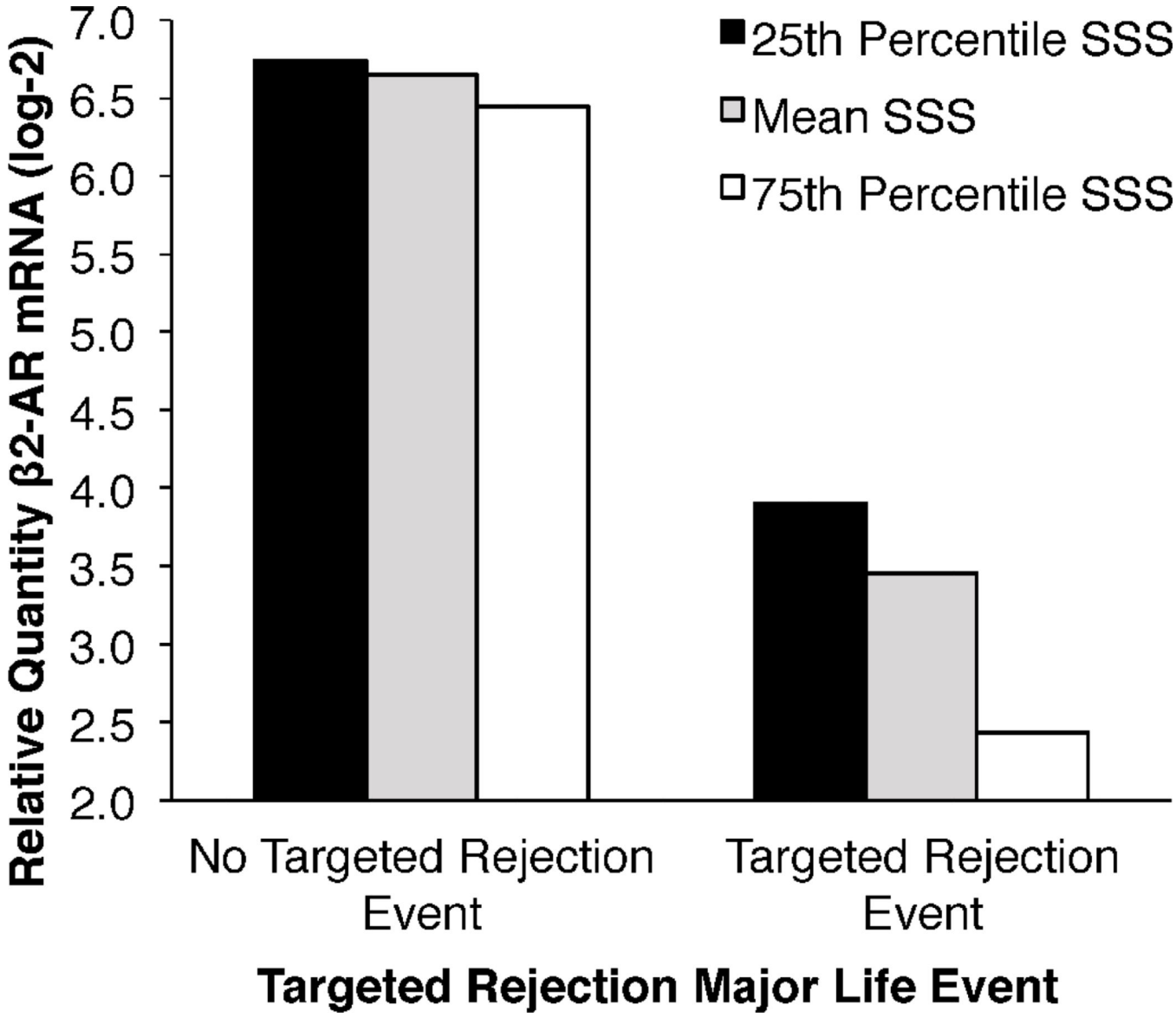

Figure 2.

Regression-based point estimates of the differences in messenger RNA (mRNA) expression for β2-adrenergic receptor (β2-AR) following the experience of a recent targeted rejection major life event (n = 17) for participants reporting different levels of subjective social status (SSS). At visits when participants had experienced a recent targeted rejection major life event, they exhibited less mRNA for β2-AR, p = .006, d = −.51. Moreover, this association was moderated by SSS, p = .05, with a simple slopes analysis revealing that youth higher in SSS showed the steepest declines in expression of β2-AR following targeted rejection, p = .001, d = − .64. Individual bars represent the point estimates (not observed means) for β2-AR mRNA based on the multilevel model regression coefficients (N = 121).

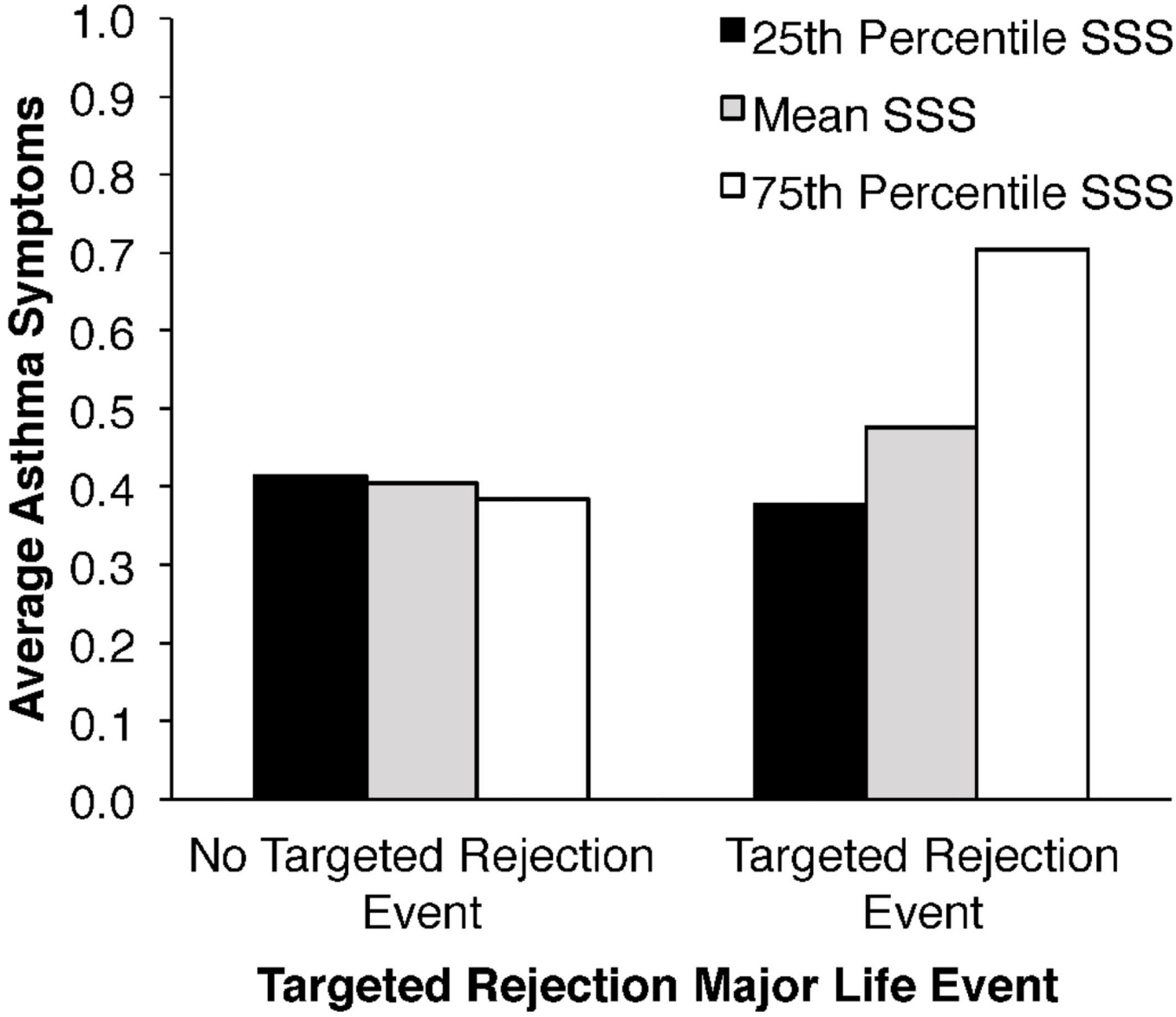

Figure 3.

Regression-based point estimates of the differences in self-reported asthma symptoms following the experience of a recent targeted rejection major life event (n = 17) for participants reporting different levels of subjective social status (SSS). As a group, at visits when participants had experienced a recent targeted rejection major life event, they did not report an increase in asthma symptoms, p = .48, d = .11. However, this association was moderated by SSS, p < .001, with a simple slopes analysis revealing that youth higher in SSS showed a significant increase in asthma symptoms following targeted rejection, p = .008, d = .47, whereas youth with lower or average SSS did not. Individual bars represent the point estimates (not observed means) for asthma symptoms based on the multilevel model regression coefficients (N = 121).

Table 1

Descriptive Characteristics of the Sample (N = 121)

Variable	*n*	%	*M*	*SD*
Age (baseline)			12.61	2.63
Gender (% male)		70		
Ethnicity (% Caucasian)		63		
Body Mass Index (BMI; kg/m^2)			21.71	4.33
Subjective social status at school (range 1 – 10)			6.60	1.48
Inhaled corticosteroids use (% of visits where use was reported within past 2 weeks)		54		
β-agonist use (% of visits where use was reported within past 2 weeks)		69		
Non-interpersonal major life events	51			
Interpersonal major life events	71			
Targeted rejection major life events	17			
GR mRNA (relative expression)			5.22	1.37
β2-AR mRNA (relative expression)			5.71	2.91
Self-reported asthma symptoms during 2-week home monitoring period following each visit (range 0 – 4)			0.31	0.40

Note: BMI, GR mRNA, β2-AR mRNA, and asthma symptoms were averaged across the 2 year study period, and life events were totaled across the 2 year study period. BMI = body mass index; GR = glucocorticoid receptor; β2-AR = β2-adrenergic receptor