Genetic Variants and the Cortisol Response in Children: An Exploratory Study

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Abstract

Objective: We examined genomic variation potentially associated with the cortisol stress response in children having a painful medical procedure. **Design:** Children 4–10 years old having a peripheral intravenous line inserted provided saliva samples for evaluation of the cortisol response as a biological measure of distress: two on the day of the procedure and two at home on a nonstressful day for comparison values. Children and biological parents also provided samples for genotyping of variants with known or suspected association with the cortisol stress response. Analysis included child-only association and family-based transmission disequilibrium tests (TDTs). **Results:** Genotype and phenotype data on the cortisol stress response were available from 326 children for child-only association analyses and 376 complete family trios for TDTs. Children were 50% female, an average of 7.5 years old, and mostly (83%) White/non-Hispanic. We identified four single-nucleotide polymorphisms (SNPs) potentially associated with the cortisol stress response: rs1176744 (*HTR3B*), rs10062367 (*CRHBP*), rs634479 (*OPRM1*), and rs8030107 (*NTRK3*). Family-based analysis identified a two-SNP haplotype in *HTR1B* suggestive for association with the cortisol response (rs6296, rs11568817). Allelic TDTs identified rs7897947 (*NFKB2*) as potentially related to cortisol response. **Conclusions:** Findings provide preliminary evidence for genes potentially important in cortisol response to an acute stressor in children in the serotonin, dopamine, and brain-derived neurotrophic factor pathways, the hypothalamic–pituitary–adrenal axis, and the inflammatory response. Combined with analyses of related phenotypes and clinical data, these results could help identify patients at increased risk of adverse responses to painful medical procedures who might benefit from tailored interventions.

Keywords

cortisol, genetic variants, procedural stress

Individuals vary widely in their psychological, behavioral, and biological responses to acute stress, with the sympathetic nervous system and hypothalamic–pituitary–adrenal axis (HPA) as primary mediators (Chrousos & Gold, 1992; Tsigos, Kyrou, Kassi, & Chrousos, 2016). Interpretation of and initial response to a stressor occurs in the brain, which then directs the broader biological response, including activation of the HPA axis, the glucocorticoid response, and subsequent cortisol release (Mahon, Zandi, Potash, Nestadt, & Wand, 2013; Tsigos et al., 2016). One of the most well-characterized elements of the biological stress response is a rise in cortisol levels 20–30 min following stressor exposure, with a subsequent return to baseline. Many factors, including genetic variation, influence the magnitude and duration of this response, leading to substantial individual differences (Li-Tempel et al., 2016). An individual's cortisol response to stress may also change over

time due to exposures to different chronic and acute stressors over the life course (McEwen & McEwen, 2015, 2016).

The typical biological response to stress, including cortisol release and return to baseline, is relatively short lived; thus,

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homeostasis is maintained (Chrousos & Gold, 1992). However, individual cortisol responses can be larger or smaller in magnitude and may also be prolonged. Prolonged and higher than normal levels of cortisol in response to a stressor can have significant effects on short- and long-term mental and physical health (Mahon et al., 2013). Lower than expected cortisol responses may reflect early-life adversity or co-occurring internalizing conditions, such as anxiety, and have implications for later health and disease risk (Lovallo et al., 2015; McGinnis, Lopez-Duran, Morelen, Rosenblum, & Muzik, 2015). Higher than expected levels in response to acute stress also reflect dysregulated emotional and physical states and can have detrimental effects on health and well-being (Chrousos & Gold, 1992).

Because variability in the cortisol response to stress has a broad impact on health, identifying factors that contribute to this variability could facilitate implementation of interventions designed to mitigate these effects (Slopen, McLaughlin, & Shonkoff, 2014). Interindividual genetic variability is a known contributor (Lovallo et al., 2017), and identifying relevant genetic variants may help identify those at higher risk of maladaptive stress responses (Mahon et al., 2013). This ability may be particularly valuable in children, providing a biological measure in addition to self- and parent-proxy reports (Jackowska, Ronaldson, Brown, & Steptoe, 2016; McCarthy et al., 2011) and a means for tailoring interventions to those at higher risk for maladaptive stress responses.

Studies of the cortisol stress response, including those in children, often rely on lab-based standardized stress tasks (e.g., the Trier Social Stress Test; Armbruster et al., 2012; Kudielka, Hellhammer, & Wust, 2009; Sheikh, Kryski, Smith, Hayden, & Singh, 2013). However, these tests might not consistently generate stress responses in young children (Gunnar, Talge, & Herrera, 2009; Kryski, Smith, Sheikh, Singh, & Hayden, 2011); naturally occurring stressors provide an alternative. In the present study, we used a stressful but planned painful medical procedure (intravenous line [IV] insertion) to evaluate the cortisol stress response in an unselected cohort of children, providing a natural framework for their responses.

The purpose of the present analysis was to identify genetic variants associated with the cortisol stress response. This study extends earlier work examining genetic factors associated with children's pain and anxiety (Ersig et al., 2017) and evaluating the contribution of child and parent factors to cortisol response to a painful procedure (McCarthy et al., 2010a).

Method

The primary study (McCarthy et al., 2010a, 2010b, 2014) determined the impact of parent-provided distraction on children's responses to a painful medical procedure (peripheral IV insertion).

Study Sample and Setting

The study was completed at three Midwestern children's hospitals; all sites provided Institutional Review Board (IRB)

approval. We identified children 4–10 years old who were scheduled for procedures requiring peripheral IV insertion from clinic rosters and sent letters describing the study to families prior to their scheduled appointments. Children enrolled in the study were most often being seen in the gastrointestinal, nephrology, endocrinology, pulmonary, or cardiology clinic (McCarthy et al., 2010b), with children at one site also having diagnostic radiology procedures. Diagnostic tests planned for children admitted to the clinic reflect the clinics in which they were seen and include endoscopies, renal scans, bronchoscopies, and pituitary stimulation tests (McCarthy et al., 2010b).

Study Procedures

In the first phase of the primary study (McCarthy et al., 2010a, 2010b), children were randomly assigned to receive either parent-provided distraction (intervention) or routine care (control). The second phase of the primary study evaluated the effectiveness of different levels of parent-provided distraction. The level of distraction was selected for each child according to predicted child distress, with distraction techniques tailored to children's preferences (McCarthy et al., 2014). In both primary study phases, researchers assessed multiple child and parent factors to identify those that predicted children's responses to IV insertion when distraction was provided. The primary study also evaluated the contribution of genetic variants to children's self-reports of pain and anxiety (Ersig et al., 2017). For DNA analysis and genotyping, children provided whole blood or buccal swabs, while parents provided saliva samples. Supplies were sent home with families to collect samples from biological parents not present in the clinic (Ersig et al., 2017; McCarthy et al., 2010a, 2010b, 2014).

In addition to self-report and observational measures of pain, anxiety, and distress, children provided four saliva samples to evaluate the cortisol stress response as a biological measure of distress using the chew-and-spit method (McCarthy et al., 2009). Of the four samples, two were obtained in the clinic on the day of the procedure and two at home on a typical day, which established baseline values for comparison. Typical nonschool days were those when the child was not experiencing similar stress to that encountered in the clinic setting, most often a Saturday. Investigators collected the time-1-clinic sample at the time of clinic arrival and the time-2-clinic sample 20–30 min after IV insertion to reflect the child's cortisol stress response to IV insertion. The two home samples were collected to provide the child's baseline cortisol values. To account for circadian fluctuations in cortisol levels, home samples were collected within 30 min of the same time as the collection time for clinic samples, with the time-1-home sample obtained at the same time of day as the time-1-clinic sample and the time-2-home sample at the same time as the time-2-clinic sample (McCarthy et al., 2009). Investigators provided supplies for home collection to families along with detailed collection instructions and prepaid mailing envelopes for sample return.

Cortisol samples were sent for analysis to an outside lab that routinely measures intra-assay coefficient of variation (CV) to

ensure reliability of the data set. As reported in previous publications, the overall CV for the primary study was 8.18% (McCarthy et al., 2009). For the present analysis, we obtained the value for each participant's cortisol stress response by calculating the ratio of the time-2-clinic to time-2-home samples (McCarthy et al., 2010a, 2010b, 2014).

Selection of Candidate Genes/Pathways

Genes analyzed included those potentially associated with children's pain, distress, and anxiety in response to peripheral IV insertion (Ersig et al., 2017) as well as those known to be or potentially relevant to the cortisol stress response. Results we report here examine those associated with the cortisol stress response. We selected a total of 34 genes and 91 single-nucleotide polymorphisms (SNPs) for analysis; detailed information on these genes and variants is provided in Supplementary Table 1. Selected SNPs had a minor allele frequency of at least .2 (Ersig et al., 2017; Sherry et al., 2001) or were known tagging SNPs. Following quality control procedures, we analyzed 86 SNPs.

Genotyping

Genotyping procedures are detailed in prior publications (Ersig et al., 2017). Briefly, we used Fluidigm 192.24 Dynamic Array integrated fluid circuits (Fluidigm Corporation, South San Francisco, CA), Taqman[®] SNP genotyping assays (Life Technologies Corporation, NY), and a fast polymerase chain reaction protocol on the FC1 cycler. Fluorescence detection results were analyzed using Fluidigm analysis software v3.1.2, with Progeny 8 (Progeny software, LLC, Delray Beach, FL) used for data management. Following quality control procedures (Ersig et al., 2017), we analyzed data from 2,160 individuals ($n = 1,037$ children, 1,123 parents) and 86 SNPs in 34 genes. In brief, we first removed samples for which genotyping failed in >5% of analyses, then we removed families with Mendelian error rates of >2%. We also removed data from SNPs with assay failure or that were out of Hardy–Weinberg equilibrium (Ersig et al., 2017).

Cortisol Stress Response Calculation

The phenotype of interest was the cortisol stress response to IV placement, calculated using the ratio of the time-2-clinic to time-2-home samples. Since the distribution of the clinic/home ratio values was not normal, we applied the natural log transformation to the data. Analysis was based on $\ln(\text{clinic}) - \ln(\text{home}) = \ln(\text{clinic/home})$.

Covariates

We identified covariates for this analysis based on prior analyses (Ersig et al., 2017; McCarthy et al., 2010a, 2010b, 2014). Of particular interest for this analysis were parent report of a child with a diagnosis of attention-deficit hyperactivity disorder (ADHD) and the effect of the intervention (parent-provided

distraction) in the primary study, which was quantified as the Distraction Coaching Index (DCI; Kleiber, McCarthy, Hanrahan, Myers, & Weathers, 2007).

Parent report of ADHD diagnosis. Parents reported whether children had been diagnosed with ADHD. ADHD diagnosis was of interest given its known effect on the cortisol stress response: Children with ADHD are more likely to have a blunted cortisol stress response compared to children without ADHD (Kariyawasam, Zaw, & Handley, 2002; King, Barkley, & Barrett, 1998).

Treatment effect. In the primary study, children were randomized to receive varying levels of distraction coaching from parents, which was quantified using the DCI. This validated measure evaluates the quality and frequency of coaching; scores reflect use of distraction, with higher scores indicating greater expertise in coaching (Kleiber et al., 2007).

Statistical Analyses

We used all available genotype and phenotype data for child-only regression analyses of genotype and the cortisol stress response. As in the primary study (Ersig et al., 2017), we completed supplementary transmission disequilibrium tests (TDTs) using genetic data from both parents and the child (family trios). These tests had greater power than the regression analyses to detect genotype–phenotype associations and removed the influence of population admixture (Abecasis, Cookson, & Cardon, 2000; Spielman & Ewens, 1996).

We used linear regression to examine the association of child genotype with cortisol stress response. The natural log of the ratio of the cortisol level in clinic following IV insertion to the comparable home cortisol level (i.e., time-2-clinic:time-2-home cortisol) was the dependent variable. As mentioned above, we used the natural log transformation to normalize the distribution of the cortisol clinic:home ratio. The primary independent variable was number of rare alleles for SNPs of interest. The model also controlled for two covariates: parent report of child diagnosis of ADHD and DCI.

To interpret the results of the regression analysis, we expressed the regression coefficient estimate (β) from the fitted regression model in the natural log scale as e^β . This corresponds to the ratio of mean cortisol responsivity (CRRatio) associated with the presence of a rare allele (i.e., mean 1 rare allele:mean 0 rare allele or mean 2 rare alleles:mean 1 rare allele). An effect size greater than 1 for a particular SNP means that each rare allele at that particular site, as determined from frequencies in European populations, generates a more substantial cortisol stress response. Conversely, effect sizes less than 1 indicate that each rare allele at that site generates a less substantial cortisol stress response. Power analysis for this study identified detectable ratios of cortisol responsivity for effect sizes <1 of at most 0.73–0.80 and for effect sizes >1 of at least 1.26–1.37 for the distribution of rare alleles identified in our study sample, with $n = 326$, at the

0.05 significance level with 0.80 power (see Supplementary Table 2).

To assess for gender differences in the effect of genotype on cortisol responsivity, we expanded the regression model to include gender and Gender $\times$ Genotype interaction effects. We report gender-specific genotype effects for SNPs where there was an indication of a Gender $\times$ Genotype interaction effect.

We used genetic data from both parents and the child to conduct TDTs. These tests examined the effect of transmission of parental alleles to children, for which untransmitted parental alleles served as a control. We performed single-marker and multimarker (haplotype) tests in PLINK v1.07 (Purcell et al., 2007).

Given the exploratory nature of our analyses, we used liberal p value thresholds to identify SNPs with possible associations with the cortisol stress response. For regression and TDT analyses, we report significant results with a p value $< .05$. For those results that failed to reach traditional levels of significance (i.e., $p < .05$), we report those SNPs with p values $< .10$, as these results may be of interest to others examining genomic variation associated with similar phenotypes. Future studies leveraging our findings and focusing on just those SNPs closest to significance can provide more power.

Results

Sample Characteristics

The total study sample was 1,116 children. However, the present analysis required (a) a DNA sample that passed quality control measures and was analyzed for the SNPs of interest and (b) two cortisol samples (time-2-clinic and time-2-home) of sufficient quality for analysis of the cortisol stress response. After we applied these parameters, we analyzed genotype–phenotype associations for the cortisol stress response for 326 children. Exact sample size for each variant depended on data available.

For the family-level TDT analysis, data were available from 626 trios. We removed trios with one or more individuals with no calls $\geq 5\%$ ($n = 219$) and those with a Mendelian error rate of $>2\%$ or more than one Mendelian error in the trio ($n = 31$), leaving our final sample as 376 trios. We subjected parents' DNA samples to the same quality control measures as their children's; because of this, the number of family trios with data available for analysis of particular haplotypes varied for each haplotype analysis.

Children were evenly split between males and females (male $n = 163$, 50%), had an average age of 7.5 ± 1.8 years (range 4–10 years), and were 83% White/non-Hispanic, mirroring the overall study sample (see Table 1; McCarthy et al., 2010a, 2010b, 2014). Children diagnosed with ADHD comprised 10% ($n = 32$) of the sample, of whom 70% were boys, and the mean DCI score, reflecting the treatment effect, was 21.0 ± 14.2 (range 0–40).

Table 1. Descriptive Statistics.

Variable	Descriptive Statistics ($N = 326$, unless otherwise indicated)
Demographic characteristics	
Gender (male), n (%)	163 (50)
Age (years), mean + SD (range)	7.5 + 1.8 (4 – 10)
Race/ethnicity, n (%)	
White/non-Hispanic	271 (83)
Hispanic	7 (2)
African American	11 (3)
Asian	6 (2)
Native American	2 (1)
Mixed	29 (9)
Potential covariates	
Difficult IV insertion, n (%)	89 (27)
Topical anesthetic within expected dwell time, n (%)	278 (87)
ADHD diagnosis, n (%)	32 (10)
Anxiety disorder diagnosis, n (%)	11 (3)
Has child ever had an IV? yes, n (%)	244 (75)
Distraction Coaching Index	
Median (25th–75th percentile)	24.9 (6.0–33.0)
Mean (SD)	0.298 (0.270)
Range	0–40
Outcome variables	
Home cortisol (mcg/dl)	
Median (25th–75th percentile)	0.196 (0.130–0.306)
Mean (SD)	0.201 + 0.155
Range	0.015–4.553
Clinic cortisol (mcg/dl)	
Median (25th–75th percentile)	0.287 (0.178–0.514)
Mean (SD)	0.298 + 0.270
Range	0.0002–6.784
Cortisol responsivity (clinic/home ratio)	
Median (25th–75th percentile)	1.45 (0.71–3.00)
Mean (SD)	1.48 + 1.67
Range	0.001–41.57

Note. ADHD = attention deficit hyperactivity disorder.

Phenotypes

The mean time-2-home cortisol value was .201 mcg/dl ($SD = 0.155$), while the mean value of the time-2-clinic sample was .298 mcg/dl ($SD = 0.270$), reflecting expected higher cortisol values post-IV insertion. We calculated the cortisol stress response as the ratio of the time-2-clinic value to the time-2-home value. The mean ratio was 1.48 (95% CI [1.31, 1.67]; $p < .0001$; $SD = 1.67$, range = 0.001–41.57).

Genotype–Phenotype Analysis

Analysis of child genotype identified four SNPs potentially associated with the cortisol stress response in children having an IV inserted (see Table 2). Having the rare alleles at rs1176744 in *HTR3B* and rs10062367 in *CRHBP* was associated with a lower cortisol stress response (rs1176744 $p = .012$, CRratio = 0.79 [0.65, 0.95]; rs10062367 $p = .016$, CRratio = 0.77 [0.62, 0.95]). In the opposite direction, having the rare

Table 2. Suggestive Results of Child-Only Regression Analysis ($p < .05$).

Gene	SNP	Rare Allele (MAF)	Cortisol Ratio p value OR (95% CI)
HTR3B	rs1176744	G (.262)	.012 0.79 (0.65, 0.95)
OPRM1	rs634479	C (.167)	.013 1.30 (1.06, 1.59)
CRHBP	rs10062367	A (.254)	.016 0.77 (0.62, 0.95)
NTRK3	rs8030107	T (.439)	.039 1.18 (1.01, 1.39)

Note. Odds ratios > 1 (bolded) indicate SNPs for which each additional rare allele generates a more substantial cortisol response to the stressor. MAF = minor allele frequency; SNP = single-nucleotide polymorphism.

allele genotypes at rs634479 in *OPRM1* and rs8030107 in *NTRK3* was associated with a greater mean cortisol stress response (rs634479 $p = .013$, CRratio = 1.30 [1.06, 1.59]; rs8030107 $p = .039$, CRratio = 1.18 [1.01, 1.39]).

Results for four additional SNPs indicated possible associations, with p values of .05–.12. Rare alleles for two SNPs were associated with a lower cortisol stress response: rs6296 in *HTR1B* and rs1143629 in *IL1B* (rs6296 $p = .089$, CRratio = 0.85 [0.70, 1.03]; rs1143629 $p = .064$, CRratio = 0.84 [0.70, 1.01]). Rare alleles at the other two SNPs generated a larger cortisol stress response: rs1876831 in *CRHR1* and rs3212363 in *MC1R* (rs1876831 $p = .119$, CRratio = 1.23 [0.95, 1.60]; rs3212363 $p = .091$, CRratio = 1.18 [0.97, 1.43]).

We also examined Gender $\times$ Genotype interaction effects to identify differences between males and females. This analysis identified two groups of SNPs for which gender-specific results differed. A difference in the direction of effect was identified for seven SNPs. Females had a more robust cortisol response for rs10478694 and rs2941026, while males had a more robust response for rs2023239, rs4563479, rs6537485, rs7668086, and rs941601. For five SNPs, we detected an effect for one gender but not the other (effect identified for males only for rs3212363 and rs6280; effect identified for females only for rs6812093, rs6821368, and rs6827096).

TDT Analysis

Results of the TDT analysis are suggestive given the number of informative trios with complete genotype–phenotype data (see Table 3). A two-SNP haplotype in *HTR1B*, for rs6296 and rs11568817 (global p value .02, 27 informative trios; CT genotype $p = .004$), had results suggesting an association with greater cortisol response, while allelic TDT analysis identified one SNP (*NFKB2*, rs7897947; $p = .02$) potentially related to greater cortisol response.

Discussion

The results of this analysis provide preliminary evidence for genes and pathways potentially important in cortisol response

Table 3. Suggestive Results of Family-Based Genetic Association Test (Transmission Disequilibrium Test; $p < .05$).

Gene	SNP	Rare Allele (MAF)	Cortisol Ratio p Value
<i>NFKB2</i>	rs7897947	G (.192)	.02
<i>HTR1B</i>	rs6296	G (.323)	Global $p = .02$ (27 inform).
	rs11568817	G (.379)	CT (31%, 24 inform, $p = .007$); CG (41.6%, 19 inform, $p = .04$, Z-score)
<i>CRH</i>	rs6982394	A (.035)	2SNP (rs4613981, rs2446432): Global $p = .04$ (29 inform).
	rs12721510	A (.05)	AG (48%, 24 inform, $p = .009$)
	rs4613981	G (.136)	4SNP: Global $p = .07$ (29 inform).
	rs2446432	G (.49)	CCAG (48%, 24 inform, $p = .008$)

Note. MAF = minor allele frequency; SNP = single-nucleotide polymorphism.

to an acute stressor in children and could guide future analyses. Our findings highlight genes in the serotonin, dopamine, and BDNF pathways; the HPA axis; and the inflammatory response to acute stress. Specific SNPs identified in this analysis differ from those reported previously, although pathways and genes of interest align with prior analyses (Alexander et al., 2014; Armbruster et al., 2009; Ketchesin, Stinnett, & Seasholtz, 2017; Mueller et al., 2011; Sheikh et al., 2013). One potential explanation for the difference in specific SNPs among studies is that our analysis included SNPs chosen for their potential relationship to other phenotypes of interest (e.g., pain, anxiety) in the primary study (Ersig et al., 2017).

In the present analysis, two genes of interest are involved in the serotonin pathway, a known contributor to the cortisol stress response (Alexander et al., 2014; Armbruster et al., 2009). While prior research focused nearly exclusively on the 5HTTLPR region of the *SLC6A4* serotonin transporter gene (Alexander et al., 2014; Mueller et al., 2011), our analysis included other genes in the pathway in addition to *SLC6A4*. While the 5HTTLPR region of the *SLC6A4* gene was not significantly associated with the cortisol stress response in our patient population, our findings support the importance of the serotonin pathway in the cortisol stress response. Specifically, we identified *HTR1B* in child-only and family-based analyses as a potentially important gene. While not statistically significant, the consistency of our results and previous analyses suggests that this particular gene should be included in future studies of the cortisol stress response.

We also found suggestive results for an SNP in *OPRM1* in the dopamine pathway. While research has not previously identified this particular variant and gene as potentially associated with the cortisol response, it has identified other genes in the dopamine pathway (e.g., *DRD3* and *DRD4*). Prior work examining genomic variation associated with the cortisol stress response identified interactions between the dopamine and serotonin pathways (Armbruster et al., 2009) and the importance of certain brain regions in the stress response (e.g., the amygdala). Exploring these relationships in future studies will support a more comprehensive understanding of the brain

structures, biological pathways, and genes and variants involved in the response to acute stress (Taylor, Larson, & Lauby, 2014). As Taylor, Larson, and Lauby (2014) highlight, “This supports and extends the foundational argument that many different genetic variants converge to influence a given stress profile, with each individual variant making a subtle yet stable contribution” (p. 6).

Additionally, we identified SNPs in *CRHBP*, a gene critical to HPA-axis function, as potentially associated with the cortisol stress response to a painful procedure. The HPA axis is an established pathway for the acute stress response in humans, and individual variation in this pathway leads to similar variability in the psychological and biological response to stress (Ketchesin et al., 2017; Sheikh et al., 2013). Corticotropin-releasing hormone (CRH) is the primary mediator of the stress response in the HPA axis; the associated binding protein, encoded by *CRHBP*, is highly conserved across species and may be particularly critical in human brain tissue (Ketchesin et al., 2017). Potential cross-reactivity with inflammatory markers highlights the importance of evaluating the role of different pathways and their interactions in the human stress response. Prior work identified an SNP in *CRHBP*, rs10473984, which was associated with cortisol reactivity in children experiencing a standardized stressor (Sheikh et al., 2013). While we identified a different SNP in *CRHBP* (rs10062367) as being associated with a naturally occurring acute stressor, this SNP is in linkage disequilibrium ($r^2 = .1911$, $p < .0001$) with the one that Sheikh and colleagues identified. Our results support the importance of *CRHBP* in determining individual response to acute stressors.

Finally, our analysis identified an SNP in *IL1B* (rs1143629) that is potentially associated with the cortisol stress response. *IL1B* mediates the body’s inflammatory response to stress (DeVon, Piano, Rosenfeld, & Hoppensteadt, 2014). Higher levels of inflammation lead to greater stress responses in animals and humans, though acute stress can also have protective effects on the immune system and inflammatory response, for example, by limiting the length of time during which different immune cells increase in number (Breen et al., 2016). The endocrine and immune systems interact during the stress response, and impaired balance and interactions between the two systems may underlie the harmful effects of chronic stress in humans (Bekhat, Rowson, & Neigh, 2017). The SNP we identified as potentially important in the cortisol stress response in our cohort was also potentially associated with observed distress, a behavioral stress response phenotype in our parent study, and self-reported pain and anxiety (Ersig et al., 2017). Overlap in the genes identified for a biological (cortisol stress response) and behavioral (observed distress) phenotype supports future work evaluating the potential role for this and other genes involved in the inflammatory response to acute stress.

Expanded use of genomic sequencing will provide extensive genomic data on members of the general population (e.g., the All of Us initiative, <https://allofus.nih.gov/>). Leveraging these data to explore genotype–phenotype relationships will help

advance our understanding of the biological underpinnings of behavioral and biological phenotypes. Combined with analyses of related phenotypes (e.g., procedural pain, distress), these results could facilitate identification of children at increased risk of adverse responses to painful medical procedures. Additionally, investigation of phenotypes in different domains (e.g., psychological, behavioral, observational, biological) in the same patient cohort will help identify overlapping and related phenotypes (MacNamara & Phan, 2016).

Studies of complex phenotypes will benefit from analysis of phenotype data from multiple sources (e.g., self-report, proxy report, observational, biological). Using data from multiple sources helps ensure appropriate definition and measurement of psychological and behavioral phenotypes, which are more difficult to define and measure than biological phenotypes (Krüger, Korsten, & Hoffman, 2017; Shelton & Martins, 2017). When possible, including a biological measure of the same or a related concept can provide support for the validity of self- or proxy reports of symptoms and phenotypes. While our results in the present study are preliminary, given our modest sample size and the number of genes and SNPs we examined, they do identify potentially important sources of genomic variation in pathways associated with other measures of the acute stress response. Our unique focus on a naturally occurring stressor in an unselected pediatric population provides additional support for prior work evaluating response to standardized, lab-based stressors (Armbruster et al., 2012; Kudielka et al., 2009; Sheikh et al., 2013).

Limitations

Results of the present study are suggestive of genotype–phenotype associations of interest and support the need for future work analyzing the association of genes and SNPs in these pathways with the acute stress response. Limitations include the modest study sample size for a genotype–phenotype analysis; the inclusion of multiple phenotypes, which dictated the selection of genes and SNPs of interest; the limited number of cortisol samples obtained; the use of a candidate-gene approach; and the absence of an independent replication population.

We have addressed the limitation of our study sample size by reporting our results as suggestive. Results did not reach significance using p values correcting for the number of associations examined, but they do support earlier work identifying genomic variation in the same pathways as important drivers of the individual acute stress response. The present study was also limited by parameters set in the parent study, for which the primary phenotype of interest was procedural pain. Researchers selected cortisol as a biological phenotype reflecting procedural pain and included it as a measure for the entire study; it was not, however, the primary phenotype of interest. Thus, we may have excluded other potentially important genes and SNPs from the present analysis. Due to clinic flow and time constraints, the number of cortisol samples that investigators could reliably obtain was limited to 2 (Hanrahan, McCarthy, Kleiber,

Lutgendorf, & Tsalikian, 2006). Because of this limitation, we were unable to complete an area under the curve analysis of cortisol response, which requires a minimum of three cortisol samples. Finally, we used a candidate-gene approach. Genomic analyses have primarily shifted to whole-exome or whole-genome sequencing and genome-wide association studies analyses, providing a more robust means of examining all known variants within genes of interest. We selected the candidate-gene approach because of the small sample size.

Implications

This analysis of a naturally occurring stressor experienced by many children contributes to research on response to painful procedures and provides preliminary information supporting the use of biological and genomic data to develop and implement interventions tailored to individual differences. While generic interventions provided to all children may support more adaptive responses to painful procedures, incorporating data unique to each child contributes to the implementation of personalized medicine and improved outcomes. Findings from the present study are particularly relevant for nurses caring for children during potentially painful procedures as well as for nurse researchers examining similar phenotypes in other populations. Precision health calls for implementation of targeted, tailored interventions designed to improve the health and well-being of individuals. By incorporating biological and genomic data into intervention development and use in the clinic, nurses have the opportunity to use intervention approaches that are most appropriate for each patient. For nurse researchers examining pain-, anxiety-, and stress-related phenotypes, our results provide important preliminary information on genetic variations associated with the cortisol response. Additional studies of similar phenotypes that incorporate variants that were already identified in studies such as this one will further improve our knowledge of the genomics of these complex phenotypes. This knowledge may be particularly relevant for children and others who experience repeated painful procedures, as they may experience sustained high stress levels.

Authors' Note

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Author Contributions

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Supplemental Material

Supplemental material for this article is available online.

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