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FKBP5 genotype and early life stress exposure predict neurobehavioral outcomes for preterm infants

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Abstract

PROBLEM: This study evaluated the relationship between stressful early life neonatal intensive care unit (NICU) experiences, genetic variation of a stress response-associated gene (*FKBP5*), and neurobehavioral outcomes.

METHOD: The impact of genetic variation and stress experience on neurobehavioral outcomes was examined for 41 preterm infants. Statistical analyses explored the main effects of *FKBP5* genotype and NICU stress experience, as well as their interaction on infant neurobehavioral development prior to discharge.

RESULTS: Statistical analyses demonstrated a relationship between both *FKPB5* genotype and stress related to NICU care that were independently associated with neurobehavioral outcomes; indicating a main effect of genotype and a main effect of stress on neurodevelopment. Additionally, we found an interaction between the minor allele genotype and NICU stress potentially associated with less favorable developmental progress at discharge.

IMPLICATIONS: Evidence of genetic and environmental risk factors for neurodevelopmental impairment suggests the need for improved evidence-based practice initiatives to protect those most vulnerable to the combination of genetic susceptibility to stress and medical fragility.

KEYWORDS
early life experience, *FKBP5*, genotype, neurodevelopment, NICU, prematurity, stress

1 | INTRODUCTION

Early life experiences are considered the developmental underpinning of physical and emotional health (Als et al., 2004; Bowlby, 1958; McGowan & Szyf, 2010). Accumulating literature supports linkages between early life experiences and lifelong health and illness (Fox, Levitt, & Nelson, 2010; Gudsnuk & Champagne, 2011; McGowan & Szyf, 2010). Infants requiring admission to the neonatal intensive care unit (NICU) may be particularly vulnerable to negative early life experiences that have the potential to result altered growth and development. Alterations include decreased post-natal body and head growth (Vinall et al., 2012), 2 times higher risk for attention deficit disorder (Scott et al., 2012), 3 times higher risk for autism spectrum disorder (Kuzniewicz et al., 2014), and 20–30% risk of developing a mental health issue (Singh, Kenney, Ghandour, Kogan, & Lu, 2013). While the NICU is focused on life saving measures, physical and social-emotional trauma experienced during care may have both acute and long-term impact on health and development (D'Agata, Young, Cong, Grasso, & McGrath, 2016; Vinall & Grunau, 2014). A vulnerable infant in the NICU encounters disruption in parental relationships, repeated stressful experiences, and pain that may be underappreciated and/or undertreated. Many of these treatment-related experiences fit the classic definition of "uncontrolled stress exposure," a circumstance that has known deleterious effects on physical, cognitive, and social-emotional health (Joels & Baram, 2009; McGowan et al., 2009; Schore, 2001). Given the unique cumulative experience associated with the NICU, we have recently proposed the term *Infant Medical Trauma in the NICU* (IMTN); a broader review of the literature and findings for stress in the NICU can be found in D'Agata, Young et al., 2016.

Traditionally, the term "trauma" has related to a physical wound however, in the 19th century an understanding of psychological trauma evolved. According to the American Psychological Association (APA), trauma is defined as an emotional response to a terrible event

(APA, n.d.). As described in the DSM-5, individuals may present trauma symptoms characterized by intrusive re-experiencing, avoidance behavior, alterations in cognition and mood, and hyperarousal resulting from traumatic experiences (APA, 2013). The traumatic experiences of premature birth and NICU care, have been described for years, yet primarily from the parents' perspective (Lasiuk, Comeau, & Newburn-Cook, 2013; Lefkowitz, Baxt, & Evans, 2010). While the parental experience is certainly important, research has largely overlooked understanding these experiences from the infants' perspective. The infant is significantly disadvantaged because they do not yet possess the behavioral or verbal repertoire to describe their experience. Despite this communication shortcoming, preterm infants respond to procedures with autonomic, hormonal, and behavioral changes. This pattern of altered reactivity is evident both acutely and persistently, particularly for infants exposed to more stress at younger gestational ages (Grunau et al., 2005; Johnston & Stevens, 1996; Salavitabar et al., 2010). These bodily "score keeping phenomena" are reflected in studies reporting that heel hyperesthesia noted by parents of former preterm infants (Abdulkader, Freer, Garry, Fleetwood-Walker, & McIntosh, 2008; Van der Kolk, 1994). While infants may be unable to verbalize their experience, there is little doubt that repeated and intense exposure to pain, stress, and parent separation are traumatic.

Stress can have beneficial effects for growth and development, however, the intensity and chronicity of stress can overburden an individual beyond their coping threshold, leading to detrimental effects. The acute stress response encourages behavioral adaptation to a challenge and mobilizes the metabolic resources to make that adaptation possible (Joels & Baram, 2009). Chronic stress though can be more destructive and has been shown to negatively impact brain structure and behavioral adaptation (Joels & Baram, 2009; Salomons et al., 2012; Smith et al., 2011; Vinall & Grunau, 2014). Vinall et al. (2014) found school age children born very preterm infants who experienced more than 74 invasive procedures during NICU care had altered white matter microstructure (Vinall et al., 2014).

Environmental factors previously shown to elicit the stereotyped stress response in NICU infants include pain, noxious stimuli, and parent-infant separation. Pain exposure often result from procedural interventions or illness states, while stress may stem from a broad array of circumstances that include elements of pain, environmental conditions, lack of protection and safety, and illness states. Pain has been shown to negatively influence long-term outcomes of former NICU infants (Brummelte et al., 2012; Ranger & Grunau, 2014; Ranger et al., 2015; Vinall & Grunau, 2014). Because preverbal infants are unable to definitively articulate painful stimuli from stressful stimuli, this study considers stress as an overarching factor inclusive of both stress and pain exposure (Coughlin, 2014).

Individual differences in the physiological response to stress are likely the result of variation within multiple genes, including those encoding the cellular and molecular aspects of stress response as well as the psychosocial reaction to stress exposure. The candidate gene *FKBP5* is a high priority candidate gene for this relationship because of its recent identification as an important functional regulator of the glucocorticoid-mediated stress response. *FKBP5* is located on chromosome 6 and encodes *FKBP5*, the FK506 binding protein 5, which plays an important role in immunoregulation as well as protein folding and trafficking. Activation of glucocorticoid receptors by cortisol results in the synthesis of *FKBP5*, which subsequently decreases glucocorticoid receptor sensitivity for subsequent cortisol binding. As a result, *FKBP5* is a powerful regulatory mechanism involved in the physiological resolution of stress effects. Single nucleotide polymorphisms (SNP) in the *FKBP5* gene are associated with differences in glucocorticoid receptor sensitivity and stress hormone system regulation. Four SNPs previously associated with variation in the impact of stressful life experiences on mental health factors were selected for inclusion in this study, rs1360780, rs3800373, rs9296158, rs9470080 (Binder, Bradley, Liu, et al., 2008; Klengel et al., 2013). The minor allele at each of these SNP locations is present in 20–30% of the population and a haplotype consisting of these four SNPs is strongly associated with the response to childhood trauma (Bevilacqua, Carli, Sarchiapone, et al., 2012).

Genetic variants associated with HPA axis dysregulation have been identified as a risk factor for stress-related psychiatric disorders (Binder et al., 2004; Holz et al., 2015), including post-traumatic stress disorder (Klengel et al., 2013). Because of the way PTSD is defined, it is has not been considered a valid diagnostic phenomenon in the infant population. Given the parallels between the NICU experience and more traditional scenarios where PTSD develops, particularly those characterized by intense and/or chronic unpredictable stressful experiences, we hypothesize that the *FKBP5* gene may play a role in conveying risk and/or susceptibility to the medical trauma associated with the NICU experience (D'Agata, Sanders et al., 2016).

Individual differences in the response to preterm birth and NICU stay has been noted in both research and clinical settings, though the factors contributing to this variation remain to be fully elucidated. Many infants exposed to the stressful early life experience of the NICU show normal neurodevelopmental status and reach milestones as expected indicating factors other than the NICU environment play a role in the negative outcomes reported. As with many outcomes of complex etiology, it is likely that the interaction of genetic susceptibility and environmental challenges shape the infant's long-term developmental course. Given the regulatory role of *FKBP5* in stress responding, we hypothesize that polymorphisms within this candidate gene will modulate the effects of stress experienced by preterm infants in the NICU (Bevilacqua et al., 2012; Mehta, Gonik, Klengel, et al., 2011). The purposes of this study were: (i) to evaluate associations between *FKBP5* genotype and neurodevelopment; and (ii) to examine the potential interaction between *FKBP5* genotype and stress experiences.

2 | METHODS

2.1 | Participants and procedure

The data for this study were collected using a prospective observational design. A cohort of preterm infants admitted to a Northeast NICU with two locations, were invited to participate. Informed parental consent was received for 45 infants born at

gestational age (GA) 26–32 6/7 weeks, from October 2014 until September 2015. The final total of 41 infants included only those infants with completed neurobehavioral assessments and successful genotyping, 4 infants did not have complete data sets. Five parents declined to participate, the most cited reason for decline being the potential for additional infant stress from the neurobehavioral examination. Infants were excluded for: (i) chromosomal or genetic anomalies; (ii) significant central nervous system abnormalities, including grade III/IV intraventricular hemorrhage; (iii) mother with a history of substance abuse during current pregnancy, and (iv) mother younger than 18 years of age. Study procedures were approved by the human subjects review boards at the hospital and university. Informed consent was obtained from all guardians of participants. No infant deaths occurred during this study.

Data related to the infant stress experience were collected from the electronic medical record for the first 21 days of NICU care using a modified Neonatal Infant Stressor Scale (NISS) (Newnham, Inder, & Milgrom, 2009), as has been used in similar studies (Cong, 2016; Xu, Walsh, & Cong, 2016, in press). Data collection for 21 days allowed us to assess a similar timeframe for all patients based on length of stay and discharge from the NICU. Infants were examined once using the NICU Network Neurobehavioral Scale (NNNS) at about 36–37 weeks post-menstrual age prior to discharge (Lester, Tronick, & Brazelton, 2004). Following enrollment, infants were sampled once for DNA during their study participation. DNA was extracted from buccal epithelial tissue using a soft cotton swab and standard protocols (Qiagen, Gentra® Puregene® Buccal Cell Kit, #158845, Valencia, CA.). The kit manufacturer recommended buccal swabbing occur at least 30 min after an oral feeding, this guideline was followed for all infants. All samples (N = 41) were genotyped for four SNPs of *FKBP5* (rs3800373, rs9296158, rs1360780, rs9470080) using predesigned TaqMan primers and universal genotyping master mix (Life Technologies, C_27489960_10, C___1256775_10, C___8852038_10, C_____92160_10). Genotyping assays were performed according to manufacturer protocol using an Applied Biosystems StepOne Plus PCR machine and ABI allelic discrimination software. To verify accuracy and call rate, 17% of the samples were duplicated, yielding 100% reproducibility.

2.2 | Measures

Maternal and infant demographic data were collected. Maternal data included: age, race, pregnancy history, and complications. Infant data included: gestational age, gender, race, birth weight, and length, mode of delivery, Apgar scores, resuscitation at delivery, SNAPPE-II score (Dammann et al., 2010; Richardson, Corcoran, Escobar, & Lee, 2001), and length of stay.

The NISS is a ranked quantitative instrument used to collect information concerning daily stress experiences of infants during care in the NICU (Newnham et al., 2009; Smith et al., 2011). These stress experiences indicate procedures performed on an infant, the instrument does not measure response. The NISS scale was originally developed in Australia, with item weighting identified by expert opinion. Data can be collected retrospectively from the infant's medical record. Common NICU interventions are ranked on a five-point scale as not stressful, a little stressful, moderately stressful, very stressful, and extremely stressful. Each attempt at a procedure is counted as one stress event. Examples of NICU care procedures and their corresponding scoring include: each intubation attempt is extremely stressful; each suctioning attempt is very stressful; diaper change is moderately stressful; and aspiration of nasogastric tube is a little stressful. Weighted NISS data were collected daily, resulting in a Total Daily Score that measures daily stress. In this study, the daily total scores were used both as a daily stress variable and as a cumulative stress variable (cumulative daily scores). The instrument allows for quantification of stress exposure however, instrument testing has not yet determined clinical significance of scores.

The NICU Network Neurobehavioral Scale (NNNS) is a standardized assessment of neurobehavioral functioning of the high-risk infant, including neurologic, behavioral, and stress signs (Lester et al., 2004; Liu et al., 2010). The assessment specifically describes an infant's level of developmental and behavioral maturation, central nervous system integrity, and stress response (Lester et al., 2004). The broad categories include neurologic items for tone and reflexes; behavioral items of state, sensory, and interactive processes; and stress/abstinence. The NNNS has been used to examine infant groups which include: healthy full-term, preterm, and prenatally drug-exposed infants (Fink, Tronick, Olson, & Lester, 2012; Montirosso et al., 2012; Sucharew, Khoury, Xu, Succop, & Yolton, 2012). Research has shown that when evaluated early in life, NNNS assessment predicts longer-term medical and behavioral outcomes (El-Dib, Massaro, Glass, & Aly, 2012; Liu et al., 2010). The exam yields 13 summary scores; while all subscales are not scored in the same direction, for most, higher scores indicate better function. The suggested age range for use of the exam is 34–48 weeks (corrected age). To allow for a standardized approach of neurobehavioral comparison, examination of neurobehavioral functioning was conducted after 35 weeks GA and prior to NICU discharge, median GA 36.2 weeks.

2.3 | Data analysis

Descriptive statistics were determined to summarize demographic characteristics and genotype frequencies within the study sample. To investigate relationships between stress (as represented by NISS Total Average Stress) and neurodevelopmental outcomes (as measured by NNNS Summary Scores) correlation analyses were conducted using Spearman's coefficient. Multiple linear regression analysis was used to explore the contribution of the candidate gene SNPs to neurobehavioral outcomes while controlling for biological predictors of health outcomes, NICU stress and early life illness severity (Grunau et al., 2013; Valeri et al., 2016). These analyses were repeated with each NNNS summary score serving as the dependent variable. In light of the small sample size, a principle component factor analysis (PCFA) was performed for data reduction (Chau et al., 2014). The PCFA included gestational age, birth weight, total average stress, and SNAPPE-II. This latent variable from the principal components analysis was used in the regression modeling process as Model 1. Genotype of each *FKBP5* SNP was added as Model 2. Finally, interaction terms

TABLE 1 Sample characteristics

Infant	
Gestational age at birth, weeks	30.1 (±2.2)
Gender, % males	53.7
Birth weight, grams	1317 (±488)
Race, %	
White	68
Other	20
Black	12
Hispanic, % no	95
Mode of delivery, % c-section	78
SNAPPE-II	15 (1 ± 4)
Total Average Stress	128 (±28)
Length of stay, days	56 (±30)
Mother	
Age	33 (±5.5)
Marital status, % married	56

N = 41; Values are mean (SD).

between the genotype and Total Average Stress variables were entered into each model in order to explore the potential modulatory role of genotype on the effects of NICU stress.

3 | RESULTS

The participants were primarily identified as white, non-Hispanic, see Table 1. The mean gestational age was approximately 30 weeks. There were slightly more male infants and the majority of were delivered via caesarean section birth. The mean age of mothers was 33 years.

Infants experienced an average of 46 daily stressful experiences. The intensity of stress varied throughout the first 21 days of life, as can be seen in Figure 1. The 21-day weighted mean stress scores for the cohort was 128.

All infants were genotyped for *FKBP5* SNPs rs3800373, rs9296158, rs1360780, and rs9470080, with respective minor allele frequencies identified as 0.46, 0.46, 0.49, and 0.51. These frequencies were slightly higher than HapMap data, CEU population as a reference (the known HapMap allele frequencies for rs3800373, rs9296158, rs1360780, rs9470080 are: 0.24, 0.27, 0.27, and 0.30), however, all genotype frequencies were in Hardy–Weinberg equilibrium. Due to the small sample size, recessive alleles were combined and genotype frequencies were compared using the following model: rs3800373 AC + CC and AA (18, 23); rs9296158 AG + GG and AA (18, 23); rs1360780 CT + TT and AA (19, 22); rs9470080 CT + TT and CC (21, 20). A correlation analysis of *FKBP5* genotype identified significant linkage disequilibrium among SNPs rs3800373 and rs9296158 (100%), rs3800373 and rs1360780 (96%), rs1360780 and rs9470080 (86%), and rs3800373 and rs9470080 (82%) ($p < 0.05$). Given the 100% linkage disequilibrium of rs3800373 and rs9296158, rs3800373 was randomly selected as the tagging polymorphism and rs9296158 was removed from all subsequent analyses.

Using Spearman correlation, we investigated the relationship between an infant's Total Average Stress experience and NNNS neurobehavioral outcomes. Significant correlations were identified for the following summary scores: Self-Regulation ($p = 0.012$), Stress-Physiological ($p = 0.046$), and Stress-GI ($p = 0.001$). The significant correlations of Total Average Stress and NNNS summary scores are presented in Table 2.

The first factor from the PCFA (eigenvalue = 3.1) was found to explain 78% of the total variance across all four variables. When controlling for biological factors, stress, and severity of illness, the regression analyses between *FKBP5* SNPs and neurobehavioral assessment revealed rs3800373, rs1360780, and rs9470080 genotype to be predictors for Excitability ($p = 0.000$) and rs3800373, and rs1360780 predicted Stress State ($p = 0.047$), see Table 3. Stress State is a component of the overall NNNS Stress/Abstinence Summary score. For these analyses, *FKBP5* genotype was combined into one factor due to the high correlation between the four SNPs, whereby reducing the opportunity for multiple testing.

When gene × environment interactions were investigated NNNS Nonoptimal Reflexes and Stress Autonomic were associated with a

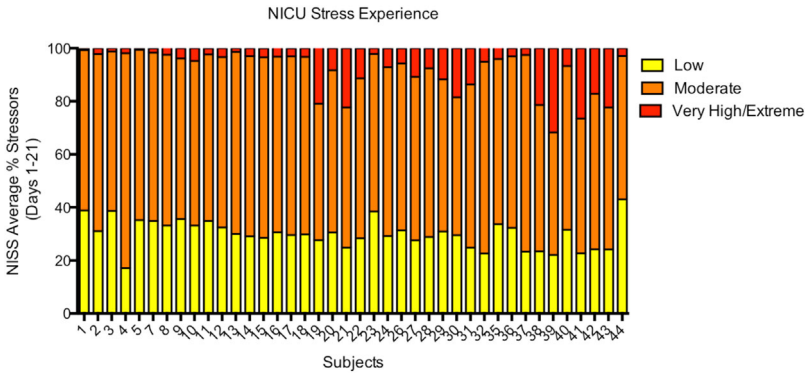

FIGURE 1 The cohort percentage of NICU stress intensity over 21 days (N = 41); Yellow = A Little Stressful; Orange = Moderately Stressful; Red = Very and Extremely Stressful. Fewer infants had high percentages of very stressful events, yet all had significant moderate and low stress experiences

TABLE 2 NNNS correlation with NISS total average stress

	Regulation	Stress physiological	Stress GI
NISS	$p = 0.012$[a]	$p = 0.046$	$p = 0.001$[a]
	r = −0.39	r = 0.31	r = 0.51

[a]Adjusting for multiple testing across three NNNS components.

significant interaction between stress experience and genotype ($p < 0.01$, $p < 0.01$), see Figure 2. For Nonoptimal Reflexes, infants with at least one copy of the minor allele had more optimal reflexes (as represented by lower scores) when in a low stress environment. However, when these infants had high stress exposure, they had higher scores for Nonoptimal Reflexes in comparison to those without the minor allele. This finding occurred across all polymorphisms in the study (rs3800373 $p < 0.01$; rs1360780 $p < 0.02$; rs9470080 $p < 0.00$), see Supplemental Table S1. The summary score of Stress Autonomic identified a similar pattern. For those infants with the risk allele at either of two locations (rs3800373 $p < 0.01$; rs1360780 $p < 0.04$) and a high stress environment, there was evidence of increased autonomic stress response relative to that experienced by infants without the risk allele. Interestingly, when infants with the minor risk allele were in a low stress environment, they actually presented with a better neurobehavioral repertoire than infants with the protective major allele.

4 | DISCUSSION

Infant outcomes following NICU care vary widely between individuals, even for infants of similar gestational ages with similar courses in the NICU; though the factors contributing to these outcomes remains unclear. The present findings indicate significant associations between NICU stress exposure, *FKBP5* genotype and neurobehavioral outcomes, showing that environmental factors act synergistically with genetic susceptibility to affect neurobehavioral outcomes. We identified two primary findings: (i) increased stress exposure in the NICU is associated with risk for poorer neurobehavioral outcomes and (ii) having at least one copy of the *FKBP5* minor allele is also associated with the risk for poorer neurobehavioral outcomes in preterm infants. This study contributes to a growing body of literature elucidating fundamental genetic susceptibility for vulnerable infants to environmental exposures (Blair, Pickler, & Anderson, 2016; Chau, Cepeda, Devlin, Weinberg, & Grunau, 2015; Chau et al., 2014; Paquette et al., 2014). Furthermore, a significant gene x environment interaction was identified between *FKBP5* genotype and stress on neurodevelopmental outcomes. This finding provides more evidence that these synergistic factors may have lasting effects on long-term outcomes. It has not been determined how the long-term the effects of increased early life stress and vulnerability from the risk allele might endure neurodevelopmentally, thus further study is needed.

The influence of *FKBP5* genotype on other brain health outcomes has been examined in limited populations. Binder et al. (2008) found that in adults who experienced childhood abuse, *FKBP5* genotype was a predictor of adult post-traumatic stress disorder (PTSD). These results are similar to other findings of an interaction between *FKBP5* minor allele risk polymorphisms with childhood adversity to modify the risk for PTSD (Mehta et al., 2011; Xie et al., 2010). In children who experienced an acute medical injury, *FKBP5* polymorphisms were associated with peritraumatic disassociation (Koenen et al., 2005). Interestingly, in study of 14-month-old infants and the quality of their parental attachment relationship, the presence of the minor allele at rs1360780 within *FKBP5* demonstrated an additive effect with cortisol reactivity such that higher cortisol reactivity was seen in infants with more risk alleles (Luijk et al., 2010). Also, an interaction was found between *FKBP5* genotype and insecure-resistant attachment. This suggests a heightened risk for *FKBP5* minor allele carriers, with either one or two copies, who also have an insecure-attachment relationship with their mother (Luijk et al., 2010). Consistent with other studies, our work provides novel evidence of a negative relationship between the *FKBP5* risk allele and neurodevelopmental outcomes in the preterm NICU infant population.

The present data suggest that while only a few NICU infants endure regular exposure to "very high/extremely" stressful events, the vast majority of infants are exposed to a remarkable number of low-to-moderate level stress events over the course of their care (see Figure 1). Furthermore, the stress experience for these infants is chronic and chronic stress has previously been shown, in preclinical and clinical research, to be detrimental to long-term outcomes (Joels & Baram, 2009). As would be expected, infants who require less supportive care, evidenced by Total Average Stress scores, had lower stress scores and better neurodevelopmental outcomes than infants who require increased medical care. Those infants with higher Total Average Scores demonstrated worse NNNS scores for self-regulation, physiological stress, and GI stress.

This study provides novel insight into the relationship between the physical stress associated with the NICU experience and polymorphisms of the stress-susceptibility gene *FKBP5*. Little research exists to elucidate the specific effects of stress in the NICU, particularly the dose and type of stressors likely to impact

TABLE 3 Regression model NNNS excitability and stress state

	Excitability			Stress state		
	F-change	R^2-change	*p*-value	F-change	R^2-change	*p*-value
Factor analysis 1	0.209	0.040	0.209	0.659	0.005	0.659
FKBP5 genotypes	0.000	0.570	0.000	0.025	0.224	0.047

Factor analysis 1 = gestational age, birth weight, total average stress, and SNAPPE-II; FKBP5 genotypes = rs3800373, rs1360780, rs94700800; N = 41.

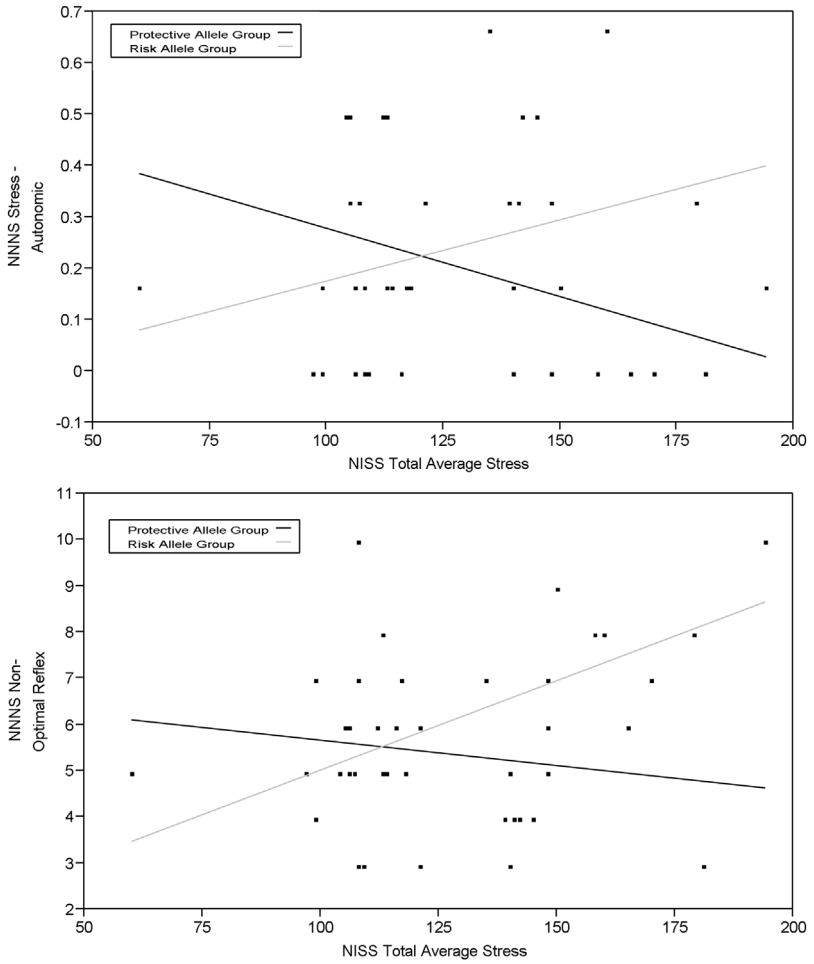

FIGURE 2 Interaction of NISS Total Average Stress and FKBP5 genotype (rs3800373) on NNNS Stress Autonomic scores ($p < 0.01$) and Nonoptimal Reflex ($p < 0.01$). As stress increases, the risk allele carriers (light gray) scores increase for both stress autonomic and non-optimal reflexes. For the protective allele group (dark gray), as stress increases their neurobehavioral scores decrease. Higher scores for both measures indicate worse findings

long-term outcomes. Moreover, little is known about the mechanisms underlying individual differences in preterm infant outcomes following extended NICU hospitalization. Elucidation of these potential relationships will allow for further exploration of gene x environment interactions and ultimately to design neuroprotective interventional strategies. The ultimate goal for research that identifies molecular differences in how individuals perceive medical care experience would be to develop evidence-based practice initiatives to protect those that are most vulnerable, due to the combination of genetic susceptibility to stress and medical fragility.

Several limitations should be considered with the present findings. First, as an exploratory genetic study the sample size is small, and thus, our ability to conduct some analyses, including haplotype analyses are restricted. Second, due to the low number of infant carriers of the minor allele, we lacked statistical power to analyze the three genotypes individually. However, others have taken this same approach of combining minor allele carriers and have had similar success in identifying genetic associations (Buchmann et al., 2014). Third, due to the nature of the NNNS summary score data, the lack of a single overall score NNNS score restricted the analyses to those that assess individual aspects of neurodevelopment rather than a global assessment. Fourth, the authors acknowledge the interaction analysis is underpowered, however, the purpose is exploratory to identify opportunities for a subsequent, confirmatory study. Lastly, it is possible that the variations in outcomes in this study attributed to *FKBP5* may in fact reflect influences of other candidate genes (Blair et al., 2016). Further research to explore this possibility is needed.

This study indicates that infant perception of stress, based upon genetic susceptibility, influences short-term neurodevelopmental outcomes. Since the NNNS is predictive of longer-term outcomes (El-Dib et al., 2012; Liu et al., 2010; Stephens et al., 2010), this work extends the emerging literature that has begun to uncover mechanisms influencing long-term brain health of very preterm infants in the NICU (Brummelte et al., 2012; Smith et al., 2011). The unique novel contribution of the present study is the evidence that the polymorphism of a gene related to hormonal stress response FKBP5 contributes to effects of early procedural repetitive stress on neurodevelopment of very preterm infants. Other gene candidates have been identified as relevant for long-term effects of invasive procedures on outcomes, for example, COMT which is related to

expression of the neurotransmitter dopamine and to pain response (Chau et al., 2014), serotonin transporter polymorphism related to long-term effects of stress in the presence of early trauma (Chau et al., 2014), and brain-derived neurotropic factor (Chau et al., 2015). It is very important that future studies have large sample sizes that are needed to address multiple genetic polymorphisms and their interactions.

While a larger sample is necessary to replicate/verify these results, preliminary findings indicate a genetic vulnerability to early life stress predictive of neurodevelopmental outcomes at NICU discharge. If confirmed, the implications for clinical practice are critical. Almost one-half of the infants in this sample carry at least one copy of the risk allele, suggesting that a large proportion of NICU infants are at increased neurodevelopmental risk. Because we do not know the genotype of infants in the NICU, infants must be cared for as though they all carry a risk allele, and, along with it, a heightened susceptibility to stress. This suggests the need for clinical practice interventions that modulate the infant experience and possibly reconsider how NICU care is delivered (D'Agata & McGrath, 2016). Increasing the usage of supportive interventions such as skin-to-skin holding, appropriate levels of light and sound, pain management, and increased integration of parents in the care delivery process must become requirements of all units.

Experiences of intense stress have been identified for older persons as situations of potential trauma, with this stress data, we too must recognize and validate the NICU infant experience as potentially traumatic (Coughlin, 2014; D'Agata, Young et al., 2016). In doing so, we open the door for the opportunity for increased research on traumatic stress of NICU infants and to deliver trauma-informed age-appropriate care (Coughlin, 2014). The implications to delivering trauma-informed NICU care include: (i) appraisal of how caregivers are education to deliver care to vulnerable infant and (ii) modification of eligibility criteria for early intervention support to include a broader population of at-risk infants. Moving forward it will be critically important to integrate into clinical practice a very clear understanding of just how influential NICU care is to the long-term outcomes of an individual. The findings of the present study add to the growing evidence that the trauma of early life experience in very preterm infants has important long-lasting effects.

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