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Polymorphic variation in the *SLC5A7* gene influences infant autonomic reactivity and self-regulation: A neurobiological model for ANS stress responsivity and infant temperament

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Abstract

Objective: To examine the impact of polymorphic variation in the solute carrier family 5 member 7 (*SLC5A7*) gene on autonomic nervous system (ANS) reactivity indexed by respiratory sinus arrhythmia (RSA) and heart rate (HR) in infants during a dyadic stressor, as well as maternal report of infant self-regulation. Given evidence of race differences in older individuals, race was specifically examined.

Methods: RSA and HR were collected from 111 infants during the still-face paradigm (SFP). Mothers completed the Infant Behavior Questionnaire-Revised short-form. Multi-level mixed effects models examined the impact of *SLC5A7* genotype on RSA and HR across the SFP. Linear models tested the influence of genotype on the relation between RSA, HR, and maternal report of infant self-regulation.

Results: *SLC5A7* genotype significantly predicted RSA stress responsivity ($\beta=-0.023$; $p=0.028$) and HR stress responsivity ($\beta=0.004$; $p=0.002$). T-allele carriers exhibited RSA suppression and HR acceleration in response to stress while G/G homozygotes did not suppress RSA and exhibited

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less HR acceleration. All infants exhibited modest RSA augmentation and HR deceleration during recovery. Race-stratified analyses revealed that White T-allele carriers drove the overall results for both RSA (β=−0.044; p=0.007) and HR (β=0.006; p=0.008) with no relation between *SLC5A7* genotype and RSA or HR in Black infants. Maternal report of infant orienting/regulation was predicted by the interaction of *SLC5A7* genotype and both RSA recovery (β=0.359; p=0.001) and HR recovery (β=−1.659; p=0.020). RSA augmentation and HR deceleration during recovery were associated with higher maternal reports of self-regulation among T-allele carriers, a finding again primarily driven by White infants.

Conclusions: Early in development, genetic contributions to ANS are evident and predict maternal report of infant self-regulation within White infants, consistent with prior literature. The lack of associations in Black infants suggest that race differences in physiological reactivity and self-regulation are emerging during the first year of life potentially providing early evidence of disparities in health risk trajectories.

Keywords

respiratory sinus arrhythmia; *SLC5A7*; acetylcholine; temperament; self-regulation; health disparities

1. Introduction

The autonomic nervous system (ANS) is one of the earliest developing stress response systems, with evidence of reactivity even *in utero*.(Monk et al., 2000) ANS reactivity indexes an individual's adaptability to the environment and reactivity patterns are differentially associated with risks for both physical and mental illness across the life span. (Boyce and Ellis, 2005; Graziano and Derefinko, 2013; Porges, 2007; Porges and Furman, 2011) The ANS response to environmental stimuli is balanced by both the sympathetic and parasympathetic nervous systems. Respiratory sinus arrhythmia (RSA) is an index of the parasympathetic regulation of cardiac function via the vagal nerve efferent projection to the sinoatrial node of the heart, which modulates heart period variability as a function of respiration. In the literature, RSA is measured at both basal levels, sometimes referred to as resting RSA, and across stressors, resulting in patterns reflecting both RSA reactivity and recovery to the challenge. Decreased resting RSA is associated with cardiovascular disease and obesity, conditions that exhibit racial health disparities and are also impacted by health risk behaviors associated with decreased self-regulation.(Masi et al., 2007; Mensah et al., 2005; Thayer and Lane, 2007; Thayer et al., 2010) ANS stress responsivity is comprised of two component parts: reactivity and recovery. Reactivity refers to the change in RSA in response to a stressor following a non-stress baseline period and recovery refers to the change in RSA after the stressor during a non-stress period. RSA suppression (i.e., a reduction in RSA) reflects the parasympathetic nervous system withdrawal from cardiac regulation, while RSA augmentation (i.e., an increase in RSA) refers to the re-engagement of the parasympathetic nervous system in cardiac regulation. Heart rate (HR) acceleration (i.e., an increase in HR) in response to stress is associated with increased sympathetic tone and may mirror changes in RSA. As early as three months of age, infants exhibit changes in both RSA and HR reactivity in response to dyadic stressors such as the Still Face Paradigm

(SFP).(Moore and Calkins, 2004). Infants who, during the stressor epoch of the SFP, either fail to suppress RSA or fail to accelerate their HR exhibit higher negative affect and lower positive affect.(Moore and Calkins, 2004) At five months of age, RSA augmentation and HR deceleration during recovery following a stressor are associated with decreased parent report of distressed and fussy infant behavior.(Bazhenova et al., 2001)

In addition to links between RSA and behavioral regulation, RSA suppression in response to stress in infancy is predictive of other health outcomes. For example, reduced RSA suppression in response to stress in infancy is associated with increased diastolic blood pressure in adolescence.(Gangel et al., 2017) In childhood, both reduced RSA suppression and reduced HR acceleration in response to stress is associated with increased BMI later in childhood.(Graziano et al., 2011) Notably, in this study, the predictive nature of ANS stress responsivity applied only to Black children, suggesting racial differences in the associations between ANS stress responsivity and later disease risk.(Graziano et al., 2011) The paucity of studies examining ANS stress responsivity with sufficient sample size to appropriately examine race differences, despite clear evidence of both a relation between RSA and cardiovascular disease and heath disparities in cardiovascular disease, highlights the need to better understand how race may moderate the relationship between ANS stress responsivity and health.(Hinnant et al., 2011; Mensah et al., 2005; Wang et al., 2005) Acknowledging this gap in the literature, it remains evident that early differences in ANS stress responsivity are implicated in later behavioral and emotional self-regulation, as well as contribute to health trajectories.

RSA reactivity is also a proposed biological pathway influencing temperament.(Fox, 1989; Huffman et al., 1998; Stifter and Fox, 1990) At three months of age, RSA suppression in response to challenge is associated with maternal reports of increased soothability and duration of orienting.(Huffman et al., 1998) At five months of age, greater basal RSA and lower HR are associated with lower maternal report of infant smiling/laughter, however only greater basal RSA is associated with increased infant negative affective reactivity.(Stifter and Fox, 1990) The relationship between early physiological regulation and temperament is hypothesized to underlie both disordered self-regulation and future mental illness.(Calkins and Fox, 2002; Degangi et al., 1991) To our knowledge no studies have tested the directionality of this relationship in infancy, however it is likely to be bidirectional.

The neural structures regulating the ANS, termed the “central autonomic network” (CAN), include the amygdala, basal forebrain, prefrontal cortex, and brainstem.(Benarroch, 1993; Porges and Furman, 2011; Thayer et al., 2009) Cholinergic innervation of, and acetylcholine neurotransmission in, the CAN is critical for parasympathetic regulation of cardiac function. Additional cholinergic pathways in the CAN include vagal nerve connections, components of the descending peripheral nervous system, and the ascending flow of information from the heart and immune system back to the CAN.(Gordan et al., 2015) This integration of the CAN and ANS underlies the development of both RSA and HR stress responsivity and subsequent self-regulation that is further shaped by early social experience.(Porges and Furman, 2011)

In addition to environmental factors, there is a significant genetic influence to both RSA and HR that is stronger earlier in development and for reactivity compared to basal levels. (Boomsma et al., 1990; De Geus et al., 2007; Snieder et al., 1997). Genes, including the solute carrier family 5 member 7 (*SLC5A7*), that regulate acetylcholine, the primary neurotransmitter for the parasympathetic branch of the ANS and a primary neurotransmitter of the CAN, likely contribute to individual variation in RSA. *SLC5A7* codes for the choline transporter 1 (CHT1), which is expressed exclusively in cholinergic neurons and regulates presynaptic choline uptake; a required step in the synthesis of acetylcholine.(Apparsundaram et al., 2000; Okuda and Haga, 2000) Functional variants of CHT1 that decrease choline uptake result in a 50% reduction of acetylcholine synthesis limiting the availability of acetylcholine for neurotransmission.(Okuda and Haga, 2000; Okuda et al., 2002) Adult carriers of the T-allele (GenBank accession number: AC009963:1; dbSNP: rs333229) of a single nucleotide polymorphism (SNP; SLC5A7:c.33415T) in the 3'UTR of the *SLC5A7* gene exhibit greater basal RSA, suggesting that T-allele carriers have increased choline uptake leading to increased acetylcholine availability for neurotransmission.(Neumann et al., 2006; Neumann et al., 2005) T-allele carriers also demonstrate lower carotid intima-media thickness and atherosclerotic plaque formation, markers of cardiovascular risk, relative to G/G homozygotes.(Neumann et al., 2012) During attentional and cognitive challenge tasks, T-allele carriers exhibit lower activity than G/G homozygotes in CAN regions, including the right dorsal amygdala, anterior cingulate cortex, and right caudate, suggesting greater cortical inhibition of these CAN regions.(Neumann et al., 2006) Considering that decreases in activation of these areas is associated with greater RSA suppression and that higher basal RSA is associated with greater RSA suppression, T-allele carriers would be predicted to exhibit RSA suppression in response to stress due to elevated levels of acetylcholine mediated cortical inhibition of CAN regions and subsequently, greater basal RSA.(Thayer et al., 2009) To date, studies have only examined the relation between *SLC5A7* genotype and basal RSA in adults. Given that genetic effects are expected to be stronger during reactivity, the absence of studies exploring *SLC5A7* genotype and RSA reactivity at any age is surprising.(Boomsma et al., 1990; De Geus et al., 2007)

To address this gap, this study tested the association between *SLC5A7* genotype and both RSA and HR stress responsivity across a dyadic stressor in four-month old infants. Due to the established role of CHT1 in choline metabolism, CAN neuronal activation, and previous associations with basal RSA, we hypothesized that the greater bioavailability of acetylcholine hypothesized for T-allele carriers would be related to both greater RSA suppression and HR acceleration during the stressor. We further tested whether *SLC5A7* genotype, RSA, and HR were related to maternal report of infant temperament. Due to evidence of racial differences in both RSA and cardiovascular disease, this study directly tested whether similar patterns existed in Black compared to White infants.(Graziano et al., 2011; Hinnant et al., 2011; Mensah et al., 2005; Wang et al., 2005)

2. Methods

2.1 Subjects

The study included 111 infants (53% female) recruited as part of a longitudinal study exploring the impact of cumulative maternal life course and prenatal stress on infant development. Recruitment of pregnant women (18–41 years) took place in prenatal and Women, Infant, and Children (WIC) clinics and from ongoing studies. Mothers were excluded if they were less than 18 years of age. Only English-speaking mothers were recruited. Infant temperament, maternal age at conception, race, infant sex, maternal education level, employment, home ownership, income, savings, government assistance status, and maternal depression were collected via maternal report on an interviewer-assisted computer survey; gestational age was collected from medical records. Due to reported relationships with infant RSA, mothers with elevated depressive symptoms (n=3), assessed by Edinburgh Postnatal Depression Scale score > 13, and infants < 36 weeks gestational age (n=1) were excluded from analyses.(Feldman, 2009; Field et al., 1988) Informed consent was obtained for all subjects and the study was approved by the University Institutional Review Board.

2.2 DNA extraction and SLC5A7 genotyping

DNA was extracted from newborn bloodspots obtained from the state newborn testing laboratory. Genomic DNA was isolated according to the manufacturer's protocol (Purelink Genomic DNA Mini kit, Invitrogen, Carlsbad, California) and eluted in 70 mL of nuclease-free water. DNA samples were evaluated for double-stranded DNA integrity and concentration by Qubit dsDNA BR assay kit (Invitrogen, Carlsbad, CA) and purity by NanoDrop-2000 (Thermo Fisher Scientific, Waltham, MA) and stored at −35°C. *SLC5A7* SNP genotype was determined using the TaqMan SNP assay (Applied Biosystems). All DNA samples were run in duplicate with known controls. Samples with inconsistent duplicates were repeated. Genotyping was done blind to other variables. Due to the low prevalence of the of the T/T homozygotes, T-allele carriers were combined for analysis, consistent with previous studies.(Neumann et al., 2006; Neumann et al., 2005)

2.3 The Still-Face Paradigm (SFP)

At four months of age, 148 mother-infant dyads completed the SFP in the laboratory. The SFP is a well-established dyadic paradigm that tests infant regulatory capacity in the setting of an unresponsive caregiver.(Bazhenova et al., 2001) The procedure included three epochs of 2.5 minutes: free play (PP1), maternal neutral face and no interaction (SF; Still Face epoch), and resumption of free play (PP2). Infants were seated in a car seat throughout the procedure, facing the mother at eye level approximately three feet away. A research assistant was present but hidden. The mother was instructed not to have physical contact with the infant but otherwise to play regularly with her infant during PP1 and PP2. If an infant displayed twenty seconds of continuous distress during any epoch, the epoch was ended. Infants who did not complete a minimum of 64 seconds in each epoch were excluded (n=37 infants). The mean duration of each epoch was: PP1: 133.84 ± 4.79 seconds, SF: 130.0 ± 14.9 seconds, and PP2: 129.3 ± 17.7 seconds.

2.4 Revised Infant Behavior Questionnaire (IBQ-R) short form

During the four month visit, mothers completed the IBQ-R short form.(Putnam et al., 2014) Previous factor analyses revealed three broad infant temperament dimensions: surgency, negative affectivity, and orienting/regulation. The orienting/regulation composite score was used for analyses and includes low intensity pleasure, cuddliness/affiliation, duration of orientation, and soothability. In the first years of life, soothability and cuddliness likely reflect the caregivers involvement in the regulation of infant reactivity.(Putnam et al., 2014) Psychometric evaluation of the IBQ-R has an internal consistency of 0.75 for orienting/regulation across all IBQ forms.(Putnam et al., 2014) A higher score on the orienting/regulation scale indicates higher modulation of infant emotional reactivity, higher reported enjoyment of novelty and being held by a caregiver, increased attention/interaction to a single object, and greater distress reduction with caregiver soothing.

2.5 Autonomic measurements

2.5.1 Heart period and heart rate (HR)—Heart period data were collected from continuous electrocardiography (ECG) recording during the SFP with James Long Company equipment. The Snap-Master Data Acquisition System (HEM Data Corp., Southfield, MI) bioamplifier was set for band-pass filtering at 0.1 and 1000 Hz; data were digitized at a sampling rate of 1000 Hz with a 12-bit analog to digital board in a laboratory computer. Following data collection, ECG signal processing and analysis was conducted offline. The rising edges of the R-waves were automatically identified with a multiple-pass, self-scaling algorithm and represented graphically for artifact identification; misidentified or missing R-waves were manually corrected. The IBIs between R-waves were then prorated to equal time intervals of 125 ms. HR was assessed in beats per minute (bpm). HR was not normally distributed and was therefore winsorized to three standard deviations from the mean and natural log transformed.

2.5.2 Respiratory sinus arrhythmia (RSA)—Prorated IBIs were detrended using a moving polynomial filter to remove slow trends from the data. RSA was estimated from power spectral analysis using a discrete Fourier transform with a 64-second Hanning window and 50% overlap between consecutive windows. Spectral power was quantified in the high frequency band (0.30–0.75 Hz), a valid frequency range for four month old infants. (Bar-Haim et al., 2000) Mean RSA values were computed for each SFP epoch. An increase in RSA (i.e., RSA augmentation) across epochs reflects parasympathetic engagement, while a decrease in RSA (i.e., RSA suppression) reflects parasympathetic withdrawal. Mean RSA values in each epoch of the SFP were not normally distributed and therefore RSA data were winsorized to three standard deviations from the mean and natural log transformed.

2.6 Socioeconomic Status (SES)

Socioeconomic status (SES) was indexed by a summary score of SES was derived from education, employment, home ownership, income, savings and government assistance status (range: 0–6). Education was scored “0” for less than high school and “1” for high school or greater. Employment was scored “0” for less than full time and “1” for full time. Home ownership was scored “0” for renting and “1” for ownership. Income was scored “0” for less

than $24,999 annual income and “1” for greater than $25,000. Savings was scored “0” for less than $500 and “1” for $500 or greater. Government assistance was scored “0” for assistance and “1” for no assistance.(Bradley and Corwyn, 2002).

2.7 Statistical analysis

Descriptive statistics characterized the sample overall, by *SLC5A7* genotype, and among Black and White infants with chi-square and t-tests. Multilevel analysis using mixed-effects linear regression models were used to produce intraclass correlation coefficients (ICC) that estimate the degree of within-individual correlation of both RSA and HR across the three SFP epochs. Multilevel modeling was conducted using randomly distributed individual-specific intercepts in the following steps for both RSA and HR independently: (Model 1) examining the impact of *SLC5A7* genotype on both infant RSA and HR patterns across the SFP; (Model 2) testing the adjusted association of *SLC5A7* genotype and both RSA and HR; (Model 3) testing the moderation of *SLC5A7* genotype and both RSA and HR across the SFP by race using the unadjusted model (i.e., Model 1); examination of race stratified analyses using the adjusted model in Black infants (Model 4) and White infants (Model 5). The adjusted multilevel model controlled for the following variables: gestational age, SES, infant sex, and the interaction of infant sex and the quadratic time factor to account for sex differences in RSA patterns (Gray et al., 2017). Due to the low prevalence of the T/T homozygotes, we performed a sensitivity analysis by repeating the adjusted model excluding T/T homozygotes. To examine the relation between RSA or HR, *SLC5A7* genotype, and maternal report of infant self-regulation, change scores were generated for RSA and HR reactivity (Δ = SF – PP1), as well as RSA and HR recovery (Δ = PP2 – SF). Generalized linear models examined the moderation of both RSA and HR reactivity and recovery by *SLC5A7* genotype to predict infant orienting/regulation. We first conducted analyses across all infants and then stratified analyses by race to examine patterns within each race. The adjusted linear model controlled for the following variables: gestational age, SES, and infant sex. All analyses were conducted using SAS version 9.4 (SAS Institute, Inc. Cary, NC).

3. Results

3.1 Demographics and descriptive statistics

One hundred and eleven infant-mother dyads completed the SFP with valid RSA, HR, and IBQ-R short-form data, as well as *SLC5A7* genotype. The sample included a majority of Black infants (55.4%) and all infants were born at 36 weeks gestational age or later (Table 1). Black mothers were younger at conception ($t=-3.19$; $p=0.002$) and had shorter gestational periods ($t=-2.82$; $p=0.006$) relative to White mothers. White mothers had higher SES than Black mothers ($t=-5.02$; $p<0.0001$). No differences in RSA, HR, or orienting/regulation were found by race.

SLC5A7 allele frequencies were 0.67 (G) and 0.33 (T). The distribution of *SLC5A7* genotype (G/G homozygotes = 49, G/T heterozygotes = 50, T/T homozygotes = 12) conformed to Hardy-Weinberg equilibrium ($\chi^2 = 0.02$; $p>0.75$) and was consistent with NCBI reported frequencies. When T-allele carriers were collapsed, sex differences in allele frequencies emerged ($\chi^2=1.139$; $p=0.027$). No other differences by *SLC5A7* genotype in

maternal age at conception, gestational age, SES, or race were detected (Table 1). The mean values and standard deviations for RSA across the SFP were 17.04 ± 8.14 (mean sec) for PP1, 15.52 ± 8.25 (mean sec) for SF, and 16.82 ± 9.74 (mean sec) for PP2. The mean values and standard deviations for HR across the SFP were 143.60 ± 12.10 bpm for PP1, 149.80 ± 11.45 bpm for SF, and 148.00 ± 13.76 bpm for PP2. No differences in any individual epoch RSA or HR or IBQ-R orienting/regulation were found by *SLC5A7* genotype.

3.2 Base model (Model 1)

RSA: A high degree of correlation within each infant for RSA across epochs was evident in the mixed effects empty model (ICC: 65.7%). RSA generally decreased across the SFP (Table 2; Model 1; β=−0.343; p=0.016) with a quadratic pattern of overall RSA suppression during the SF epoch relative to PP1 and subsequent RSA augmentation during PP2 relative to the SF epoch (β=0.089; p=0.012). Although no main effect of *SLC5A7* genotype was found on infant RSA, the quadratic change of RSA across the SFP was associated with *SLC5A7* genotype (β=−0.019; p=0.060).

HR: HR estimates were highly correlated within each infant (ICC: 71.0%). HR generally increased across the SFP (Table S1; Model 1; β=0.127; p<0.0001) with a quadratic pattern of overall HR acceleration during the SF epoch relative to PP1 and subsequent HR deceleration during PP2 relative to the SF epoch (β=−0.030; p<0.0001). No main effect of *SLC5A7* genotype was found on HR, however the quadratic change of HR across the SFP was associated with *SLC5A7* genotype (β=0.003; p=0.012).

3.3 Adjusted model (Model 2)

The adjusted model for both RSA and HR accounted for race, gestational age, SES, and infant sex; infant age was examined and did not contribute to models.

RSA: *SLC5A7* genotype significantly interacted with quadratic time to predict RSA (Figure 1A; Table 2; Model 2; β=−0.008; p=0.028). T-allele carriers suppressed RSA in the SF epoch relative to PP1 and exhibited a modest RSA augmentation during PP2 relative to the SF epoch (Figure 1A). G/G homozygotes exhibited little change in the slope of RSA across the SFP.

HR: The interaction of *SLC5A7* genotype and quadratic time significantly predicted HR (Figure 1D; Table S1; Model 2; β=0.004; p=0.002). T-allele carriers exhibited HR acceleration during the SF epoch relative to PP1, while G/G homozygotes exhibited less HR acceleration during the SF epoch relative to PP1. Both T-allele carriers and G/G homozygotes exhibited a modest HR deceleration during PP2 relative to the SF epoch (Figure 1D).

Sensitivity analyses: In the sensitivity analysis, T/T homozygotes were excluded and the interaction of *SLC5A7* genotype and quadratic time remained significant for both RSA (β=−0.026; p=0.015) and HR (β=0.004; p=0.003).

3.4 Moderation by race (Model 3)

RSA: The interaction between *SLC5A7* genotype, quadratic time, and race revealed a significant three-way interaction (Table 2; Model 3; β=−0.041; p=0.042), therefore analyses stratified by race were conducted.

HR: The interaction between *SLC5A7* genotype, quadratic time, and race was not significant (Table S1; Model 3; β=0.002; p=0.47).

3.4.1 Within Black infants (Model 4)

RSA: No influence of *SLC5A7* genotype on RSA was observed (Figure 1B; Table 2; Model 4; β=0.001; p=0.94), and overall, Black infants tended to suppress RSA across the SFP (β=−0.325; p=0.064) with no RSA augmentation during recovery (β=0.059; p=0.21).

HR: No relationship between *SCL5A7* and quadratic time on HR (Figure 1E; Table S1; Model 4; β=−0.001; p=0.14) was observed.

3.4.2 Within White infants (Model 5)

RSA: *SLC5A7* genotype significantly interacted with quadratic time to predict RSA, where T-allele carriers exhibited greater RSA suppression relative to G/G homozygotes during the SF epoch relative to PP1 (Figure 1C; Table 2; Model 5; β=−0.044; p=0.007). Both T-allele carriers and G/G homozygotes exhibited RSA augmentation during PP2 relative to the SF epoch.

HR: HR was significantly and positively associated with SES (Table S1; Model 5; β=0.012; p=0.031). SLC5A7 genotype significantly interacted with quadratic time to predict HR (Figure 1F; β=0.002; p=0.008), where T-allele carriers exhibited reduced HR across the SFP relative to G/G homozygotes. T-allele carriers also exhibited greater HR acceleration during the SF epoch relative to PP1; both T-allele carriers and G/G homozygotes exhibited a modest HR deceleration during PP2 relative to the SF epoch.

3.5 Infant temperament

There was no main effect of *SLC5A7* genotype, RSA/HR reactivity, or RSA/HR recovery on the IBQ orienting/regulation scale. The adjusted model controlled for the following variables: gestational age, SES, and infant sex.

RSA: *SLC5A7* genotype significantly interacted with RSA recovery (i.e., RSA during PP2 relative to the SF epoch), but not RSA reactivity (i.e., RSA during the SF epoch relative to PP1), to predict maternal report of infant orienting/regulation (Table 3; Model1; β=0.359; p=0.001). In T-allele carriers, RSA augmentation during recovery was associated with higher infant orienting/regulation (Figure 2A). G/G homozygotes exhibited an inverse trend where RSA augmentation during recovery was associated with lower infant orienting/regulation. Race-stratified analyses revealed that White infants drove the relationship (Figure 2C; Model 3; β=0.368; p=0.004), however analyses within Black infants suggested an effect in a similar direction (Figure 2B; Model 2; β=0.313; p=0.095).

HR: *SLC5A7* genotype interacted with HR recovery (i.e., HR during PP2 relative to the SF epoch) to predict infant orientating/regulation (Table S2; Model 1; β=−1.659; p=0.020). In T-allele carriers, HR deceleration during recovery was associated with higher infant orienting/regulation (Figure 2D). G/G homozygotes exhibited an inverse trend where HR deceleration during recovery was associated with lower infant orienting/regulation. Race-stratified analyses did not reveal significant differences within Black infants (Figure 2E; Table S2; Model 2; β=−1.707; p=0.11) or White infants (Figure 2F; Table S2; Model 3; β=−0.785; p=0.42).

4. Discussion

This study tested the contribution of *SLC5A7* genotypic variation to individual differences in infant ANS stress responsivity and temperament with specific exploration of race differences in order to better understand how individual differences in infancy may contribute to health disparities later in life. Both Infant RSA and HR were significantly influenced by *SLC5A7* genotype. In response to a dyadic stressor, T-allele carriers exhibited greater RSA stress responsivity, including both RSA suppression during the stressor and modest RSA augmentation during recovery, compared to G/G homozygotes. G/G homozygotes were overall less reactive, suggesting that infants with this genotype have reduced parasympathetic regulation of cardiac function. T-allele carriers also exhibited a more reactive HR profile across the SFP, demonstrating HR acceleration in response the stressor, concurrent with the observed RSA suppression in T-allele carriers. Consistent with previous studies in adults, these data suggest T-allele carriers have increased acecytlcholine levels permitting greater cortical inhibition of CAN regions during stress and enhanced balance of sympathetic and parasympathetic regulation.

Given our interest in health disparities and the inclusion of a significant percentage of Black participants by study design, we directly tested for race differences. Despite the absence of race differences in allele frequencies, racial differences in the patterns of RSA and HR stress responsivity by *SLC5A7* genotype were found. While no effect of *SLC5A7* genotype was found in Black infants, in White infants T-allele carriers exhibited a significantly more reactive profile than G/G homozygotes, accounting for the overall impact in the full model. While this association in White infants is consistent with a previous report in White adults where T-allele carriers exhibited greater basal RSA compared to G/G homozygotes, racial differences in relation to RSA are not unprecedented.(Cushman et al., 2000; Neumann et al., 2005) For example, reduced RSA suppression is predictive of BMI and pediatric obesity only in Black children and not in White children.(Graziano et al., 2011) Racial differences in the relative contribution of genetic compared to environmental influences on the developing ANS is one hypothesis for these observed differences. For example, the absence of association in Black infants may reflect uncaptured differential environmental exposures between Black and White infants that either heightened (in Whites) or obscured (in Blacks) genetic influences.(Wang et al., 2005) An alternative explanation is that genotype may confer differential risk between Black and White individuals, a pattern found in a previous study in preschool children in which the same SNP in *COMT* resulted in *increased* risk for PTSD in White children but *decreased* risk in Black children.(Humphreys et al., 2014) To date, few studies have examined genetic associations with RSA reactivity and existing

studies likely insufficiently consider how race, sex, or developmental timing might shift the relative contribution of genetic variation to phenotypic outcomes.(Wiggins et al., 2014)

Both RSA and HR recovery interacted with *SLC5A7* genotype to predict infant orienting/regulation. T-allele carriers who, during recovery, demonstrated RSA augmentation or HR deceleration had higher maternal reports of infant orienting/regulation, consistent with the hypothesis that these infants are both distressed by maternal unresponsiveness but able to utilize maternal interaction for emotion regulation. This is a pattern reflective of the existing literature linking RSA and infant temperament.(Calkins and Fox, 2002; Feldman, 2009; Fox, 1989; Stifter and Fox, 1990) G/G homozygotes exhibited the opposite pattern; RSA suppression during recovery was associated with higher maternal reports of infant orienting/regulation. Although this interaction was stronger in White compared to Black infants, the directionality was similar in both races. Taken with the general flatter pattern of RSA across the SFP in G/G homozygotes, this suggests G/G homozygotes are overall less reactive to maternal behavior- both her unresponsiveness and her re-engagement. As such, T-allele carriers may be more receptive to maternal buffering.

Our results suggest that genotypic variation influences infant physiological and behavioral responsivity to the early social environment, specifically the dyadic parent-child relationship. Low reactive infants (e.g. G/G homozygotes) appear to have reduced capacity for engagement but also less reactivity to dyadic stressors.(Boyce and Ellis, 2005) In the context of the differential susceptibility model, G/G homozygotes may be less reactive to the caregiving environment, resulting in an advantage in low maternal caregiving environments, yet because of decreased receptivity to maternal behavior, benefit less from enriched maternal caregiving. Future analyses that examine both objective behavioral and physiological reactivity are needed to address these intriguing questions.

The plasticity of the developing ANS and the impact of *SLC5A7* genotype on RSA and HR suggest that, early in development, genetic differences influence both an infant's need for maternal support and receptivity to her buffering. These early differences likely contribute to differential risk trajectories that are further modified by the caregiver's adaptability to each infant, ideally providing a balance of external support for the infant's developing self-regulatory skills that matches the child's temperament. This early matching of temperament, physiological regulation, and caregiving likely influences subsequent risk for both psychopathology and physical health conditions.(Porges and Furman, 2011; Thayer et al., 2010) Notably, reduced basal RSA in adults is associated with modifiable risk factors of morbidity and mortality, including smoking, obesity, and physical activity, factors linked to psychopathology and impacted by self-regulation.(Thayer and Lane, 2007; Thayer et al., 2010)

Genotype-dependent differences in ANS stress responsivity in infancy also have clinical implications. The early plasticity of the ANS provides a unique opportunity to buffer adverse health trajectories for at–risk children through contingent and responsive early caregiving. This may be achieved by promoting an infant's ability to utilize maternal support for physiological regulation through the development of parent-infant co-regulation. As the infant develops, effective self-regulation can be internalized into self-initiated regulatory

mechanisms and enhanced attentional skills that are critical for later socioemotional development when the contribution of the caregiver is less prominent.(Feldman, 2007) Future research should explore how early caregiving interventions, particularly for at-risk infants, influence ANS stress responsivity and how these biological changes mediate or moderate intervention effects. Consistent with the precision medicine initiative, studies should also examine whether genotype influences both short and long-term treatment outcomes.(Keers et al., 2016) Genetic differences may contribute to variation in the influence of the early maternal-child relationship on the developing infant's stress response systems and subsequent behavioral constructs influenced by stress reactivity. These early-emerging individual differences may identify infants who require increased maternal responsivity to guide appropriate development of self-regulatory skills, as well as infants that are likely to be less responsive to dyadic interventions.

Despite the strengths of this study, limitations exist including a non-probability sample of mothers and infants recruited from one geographic region, thereby limiting external validity. The number of infants within each genotype, particularly for race stratified analyses, was small. This sample size limits within race generalizability of *SLC5A7* genotypic influence on ANS stress responsivity and precludes race by sex analyses; future studies with larger sample sizes are needed. This is, to some extent, mitigated by the consistency of our findings with previous studies and the use of repeated measures within individuals as there were 64 Black infants with 183 RSA observations and 50 White infants with 150 RSA observations. (Neumann et al., 2005) *Riese et al*, in a larger adult cohort, failed to find an association between this SNP and basal RSA, as such consideration of study population, basal compared to reactive RSA, and developmental stage is needed for future studies seeking to replicate existing findings.(Riese et al., 2014) Respiration rate, which was not assessed in the study, has been shown to influence RSA measurement, especially in adults during paced breathing or physical exercise. However, RSA measurement derived from spectral analysis, as was done in this study, is less sensitive to changes in respiration rate.(Grossman and Taylor, 2007) As a function of initial study design, a baseline assessment of RSA was not collected. A baseline measurement of RSA would allow for interpretation of the influence of *SLC5A7* genotype on both resting RSA and RSA in response to dyadic stress. The lack of a baseline RSA measurement is partially mitigated by the use of the multilevel modelling strategy that nests repeated RSA measurements within the individual. This allows for mean RSA values (i.e., the intercept) between infants to be accounted for while modeling RSA stress responsivity across the SFP. Infant sex and RSA stress responsivity interacted at a trend level and post-hoc exploratory graphical analyses, limited by sample size and power, suggests that males exhibit a less reactive pattern than females. As with the overall findings of the study, White infants drove sex differences in RSA stress responsivity. Replication in larger studies, particularly in light of race and potential sex differences, is needed. The functional significance of *SLC5A7* SNP (rs333229) is unknown; however, the known function of the gene coupled with established expression of CHT1 in CAN regions provides support for the model that variability in CHT1 is relevant for individual differences in RSA. Since the SNP is located in the 3' UTR, it is not expected to alter the amino acid sequence; however, variation in the 3'UTR may influence mRNA expression levels and/or posttranslational processing. As no evidence of functional differences for this SNP exist, it is also possible

that this SNP resides in linkage disequilibrium with another, functional, variant within the *SLC5A7* gene. A functional SNP in this gene does exist (*SLC5A7* I89V); however, the distribution of the minor allele was too low to be tested.(Okuda et al., 2002) Despite the lack of functional significance for this SNP, the integration into our hypothesis of a neurobiological model coupled with evidence in other studies that *SLC5A7* genotypic variation is associated with basal RSA and preclinical measures of hypertension diminishes the potential for false positive findings. To account for the low prevalence of T/T homozygotes, we performed a *post hoc* sensitivity analysis excluding T/T homozygotes, which did not influence findings. Recognizing that a myriad of neurotransmitters are involved in the modulation of CAN activity, especially the role of glutamatergic signaling in the cortical inhibition of CAN regions, future studies leveraging a neurogenetically informed model that assess multiple SNPs within the neurotransmission pathways influencing the ANS are needed.(Bogdan et al., 2013) Another limitation is that maternal report was used to measure infant self-regulation and not observational data. Also, assessment of maternal sensitivity and other maternal behaviors, as well as infant distress and activity level during the SFP, were not available. Studies addressing these findings integrating observational coding measures of the SFP are appropriate next steps. While we focused our analyses on maternal report of infant regulation given our initial hypothesis, previous studies have also examined infant negativity. Analyses using this subscale revealed the same patterns for both HR and RSA (data not shown).(Van den Bergh et al., 2017) We did not collect data on maternal lifetime psychopathology, *SLC5A7* genotype, or maternal physiological measures, however we did excluded mothers with high depressive symptoms at the time of the SFP to reduce potential confound. It is interesting to consider maternal *SLC5A7* genotype and ANS stress responsivity as it relates to infant ANS stress responsivity and future studies including maternal genotype and maternal-infant autonomic synchrony are needed. Finally, we did not collect data on family history of cardiovascular disease, which may influence ANS patterns.

This is the first study to identify altered RSA and HR stress responsivity profiles as a function of *SLC5A7* genotype in any age individuals and the first to observe race-specific effects. We expand the existing literature linking RSA to infant temperament and suggest that genotype is relevant. From a clinical perspective, genotype-dependent differences in ANS reactivity and recovery profiles that are associated with infant temperament suggest that individual differences in self-regulation are, in part, driven by intrinsic genetic factors. Further, the ability of early caregiver to shape the development of this system may also vary based on the intrinsic characteristics of the child. Our results provide data to support the role of acetylcholine levels and the associated neural pathways for the effectiveness of dyadic based prevention and/or intervention efforts seeking to promote increased self-regulation in at-risk infants.

5. Conclusion

We found that *SLC5A7* genotype predicted infant RSA and HR across a dyadic stressor, whereby T-allele carriers exhibited RSA suppression and HR acceleration in response to stress while G/G homozygotes exhibited little overall RSA or HR reactivity. Race-stratified analyses revealed that White infants drove both the RSA and HR reactivity findings by *SLC5A7* genotype. To complement the physiological self-regulation findings, we found that

SLC5A7 genotype interacted with both RSA and HR recovery to predict infant behavioral self-regulation. These findings add to the increasing literature linking the ANS, self-regulation, and genetics to individual differences in long-term health trajectories originating early in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *SLC5A7* genotype predicted RSA and HR reactivity across a dyadic stressor in four month old infants
- T-allele carriers exhibited RSA suppression and HR acceleration in response to stress and little recovery, while G/G homozygotes demonstrated little overall reactivity.
- Race stratified analyses revealed that White T-allele carriers drove the overall results as *SLC5A7* genotype was not predictive of RSA or HR in Black infants.
- *SLC5A7* genotype interacted with RSA and HR recovery to predict maternal report of infant orientating/regulation, where RSA augmentation and HR deceleration during recovery was associated with higher maternal reports of self-regulation only among T-allele carriers.
- Race stratified analyses revealed that White infants drove the overall temperament findings.

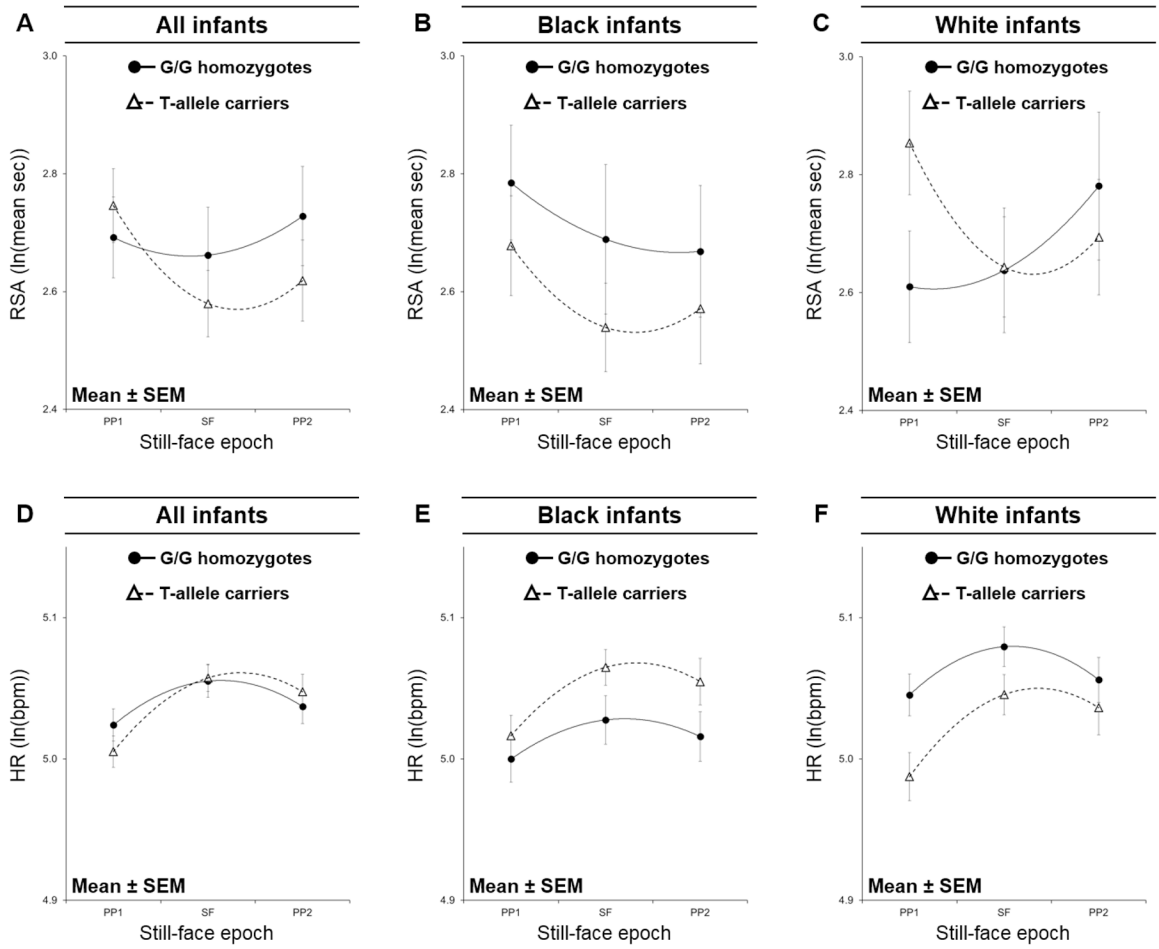

Figure 1: ANS reactivity across the SFP by *SLC5A7* genotype and race

Dot plots fitted with quadratic trend lines are presented for RSA for all infants (N=111) (A) and stratified by race, with Black infants (B; n=61) and White infants (C; n=50) presented separately. HR is reported for all infants (D) and stratified by race, with Black infants (E) and White infants (F) presented separately. Covariates in the models included gestational age, SES, infant sex, and the interaction of infant sex and the quadratic time factor.

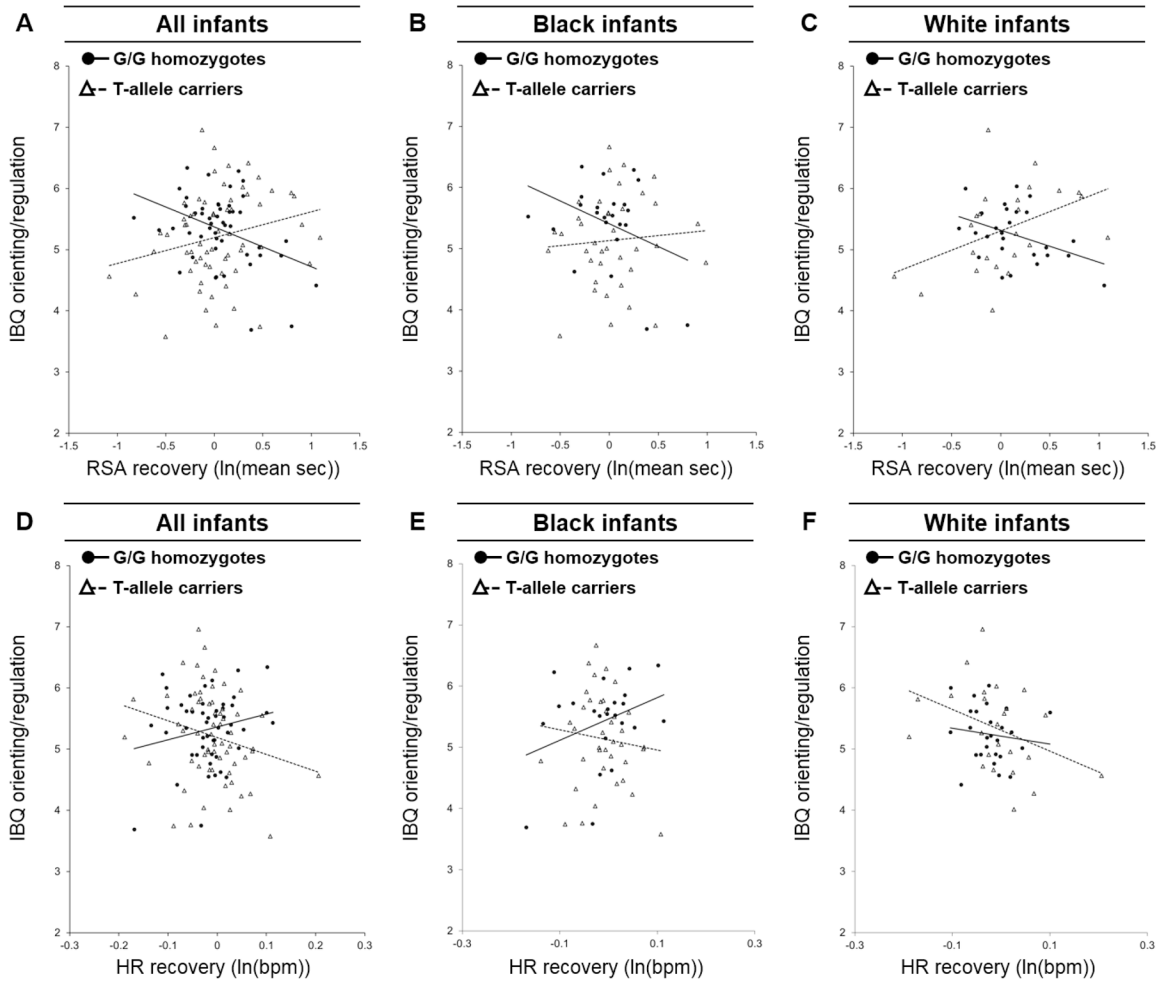

Figure 2: The moderation of ANS recovery and infant orienting/regulation score by *SLC5A7* genotype

Scatter plots fitted with linear trend lines are presented for RSA for all infants (N=111) (A) and stratified by race, with Black infants (B; n=61) and White infants (C; n=50) presented separately. HR is reported for all infants (D) and stratified by race, with Black infants (E) and White infants (F) presented separately. Covariates in the models included gestational age, SES, and infant sex.

Table 1.

Demographic characteristics and relevant covariates of study participants

Demographic outcome, mean (SD)	**Total (N=111)**	**G/G homozygotes 44.14% (49)**	**T-allele carriers 55.86% (62)**	**P-value**
Maternal conception age (yrs)	27.83 (6.01)	28.61 (6.18)	27.21 (5.85)	0.22
Gestational age (wks)	39.36 (1.30)	39.47 (1.29)	39.27 (1.31)	0.41
SES	2.97 (1.99)	3.14 (2.11)	2.84 (1.89)	0.43
	% (n)	% (n)	% (n)	
Maternal race				0.13
Black	54.95 (61)	46.94 (23)	61.29 (38)	
White	45.05 (50)	53.06 (26)	38.71 (24)	
Infant sex				**0.021**
Male	46.85 (52)	59.18 (29)	37.10 (23)	
Female	53.15 (59)	41.82 (20)	62.90 (39)	

Table 1: Groups were compared using *t* tests or chi-squared tests where appropriate.

Table 2.

Multilevel model results for the influence of *SLC5A7* genotype on infant RSA

	Model 1: *SLC5A7* genotype		**Model 2: Adjusted model**		**Model 3: Moderation by race**		**Model 4: Black infants**		**Model 5: White infants**	
Sample size	**111**		**111**		**111**		**61**		**50**	
	β	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
Time	**−0.323**	**0.016**	**−0.343**	**0.015**	**−0.343**	**0.015**	−0.325	0.064	−0.364	0.11
Time^2	**0.089**	**0.012**	0. 066	0.086	**0.070**	**0.050**	0. 059	0.21	0.067	0.27
***SLC5A7* genotype**										
G/G homozygotes	Ref		Ref		Ref		Ref		Ref	
T-allele carriers	0.042	0.67	0.061	0.56	−0.128	0.35	−0.140	0.34	0.259	0.081
Time^2 × *SLC5A7* genotype										
Time^2 × G/G homozygotes	Ref		Ref		Ref		Ref		Ref	
Time^2 × T-allele carriers	**−0.019**	**0.060**	**−0.023**	**0.028**	0.002	0.88	0.001	0.94	**−0.044**	**0.007**
Race			0.062	0.54	−0.204	0.17				
Time^2 × Race					**0.036**	**0.017**				
***SLC5A7* genotype × Race**										
Race × G/G homozygotes					Ref					
Race × T-allele carriers					0.364	0.071				
Time^2 × *SLC5A7* genotype × Race										
Time^2 × Race × G/G homozygotes					Ref					
Time^2 × Race × T-allele carriers					**−0.041**	**0.042**				
Infant sex			−0.045	0.66			0.038	0.80	−0.126	0.40
Time^2 × infant sex			0.017	0.097			0.005	0.72	**0.032**	**0.048**
SES			−0.003	0.91			−0.006	0.87	−0.012	0.72
Gestational age			0.004	0.92			−0.030	0.59	0.034	0.47

Table 3.

Generalized linear model results for the moderation of infant orienting/regulation and RSA recovery by *SLC5A7* genotype

	Model 1: All infants		**Model 2: Black infants**		**Model 3: White infants**	
Sample size	**111**		**61**		**50**	
	β	**P-value**	**β**	**P-value**	**β**	**P-value**
Intercept	**5.022**	**0.009**	4.47	0.14	**5.599**	**0.011**
***SLC5A7* genotype**	−0.055	0.19	−0.093	0.14	−0.003	0.96
RSA recovery	**−1.023**	**0.005**	−1.110	0.073	−0.877	0.037
***SLC5A7* genotype × RSA recovery**	**0.359**	**0.001**	0.313	0.095	**0.368**	**0.004**
Race	0.095	0.49				
Infant sex	0.012	0.93	0.037	0.85	0.007	0.96
SES	−0.015	0.66	0.023	0.68	−0.039	0.32
Gestational age	0.010	0.84	0.024	0.76	−0.003	0.95