# Reduced Frontal Gamma Power at 24 Months is Associated With Better Expressive Language in Toddlers at Risk for Autism

Carol L. Wilkinson, April R. Levin, Laurel J. Gabard-Durnam, Helen Tager-Flusberg, and Charles A. Nelson

Frontal gamma power has been associated with early language development in typically developing toddlers, and gamma band abnormalities have been observed in individuals with autism spectrum disorder (ASD), as well as high-risk infant siblings (those having an older sibling with ASD), as early as 6 months of age. The current study investigated differences in baseline frontal gamma power and its association with language development in toddlers at high versus low familial risk for autism. Electroencephalography recordings as well as cognitive and behavioral assessments were acquired at 24 months as part of prospective, longitudinal study of infant siblings of children with and without autism. Diagnosis of autism was determined at 24–36 months, and data were analyzed across three outcome groups—low-risk without ASD ($n$ = 43), high-risk without ASD ($n$ = 42), and high-risk with ASD ($n$ = 16). High-risk toddlers *without* ASD had reduced baseline frontal gamma power (30–50 Hz) compared to low-risk toddlers. Among high-risk toddlers increased frontal gamma was only marginally associated with ASD diagnosis ($P$ = 0.06), but significantly associated with reduced expressive language ability ($P$ = 0.007). No association between gamma power and language was present in the low-risk group. These findings suggest that differences in gamma oscillations in high-risk toddlers may represent compensatory mechanisms associated with improved developmental outcomes. ***Autism Res*** *2019, 00: 1–14.* © 2019 International Society for Autism Research, Wiley Periodicals, Inc.

**Lay Summary:** This study looked at differences in neural activity in the gamma range and its association with language in toddlers with and without increased risk for ASD. At 2 years of age, gamma power was lower in high-risk toddlers without ASD compared to a low-risk comparison group. Among high-risk toddlers both with and without later ASD, reduced gamma power was also associated with better language outcomes, suggesting that gamma power may be a marker of language development in high-risk children.

**Keywords:** electroencephalography (EEG); language; infants; cognitive neuroscience; children

## Introduction

Autism spectrum disorder (ASD) is defined by (a) deficits in social communication or interaction, and (b) restricted or repetitive behaviors [American Psychiatric Association, 2013]. However, individuals with ASD are remarkably heterogeneous in their phenotype—both in the presentation of core symptoms, as well as associated key developmental milestones such as language and cognitive development. Furthermore, language development of toddlers diagnosed with ASD can be quite variable, with 30% being minimally verbal by school age, and roughly one-quarter developing age-appropriate expressive language skills [Anderson et al., 2007; Tager-Flusberg & Kasari, 2013]. In fact, language acquisition by the end of preschool is a strong predictor of later achievement and functioning [Billstedt, Gillberg, & Gillberg, 2005; Gotham, Pickles, & Lord, 2012; Miller et al., 2017; Szatmari et al., 2009]. As such, it is important to identify early brain factors that not only influence the development of the core symptoms in ASD, but also impact language development.

A goal in improving the functional outcomes of children with autism is to identify those at greatest risk as early in life as possible, often before the behavioral repertoire of the infant is sufficiently mature to reveal consistent signs of the disorder. Infant siblings of children with ASD have an increased incidence of ASD diagnosis, currently estimated to be as high as 1 in 5 [Ozonoff et al., 2011], and are also at increased risk for language delay [Marrus et al., 2018]. Earlier identification of potential language delay in toddlers at risk for ASD would allow for earlier intervention and possibly improve outcomes. In this context, a great deal of recent attention has been paid to recording the brain's electrical activity using

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electroencephalography (EEG) from infant siblings as a way of identifying clinical biomarkers for both future ASD diagnosis but also for comorbidities such as language delay [Elsabbagh & Johnson, 2010; Gabard-Durnam, Tierney, Vogel-Farley, Tager-Flusberg, & Nelson, 2015; Jones, Venema, Lowy, Earl, & Webb, 2015; Tierney, Gabard-Durnam, Vogel-Farley, Tager-Flusberg, & Nelson, 2012].

EEG measured gamma band power (~30–80 Hz) is of particular interest in both language development and ASD as gamma activity has been associated with higher order cognitive processes including sensory integration, as well as information and language processing [Benasich, Gou, Choudhury, & Harris, 2008; Engel & Singer, 2001; Gou, Choudhury, & Benasich, 2011; Peña, Pittaluga, & Mehler, 2010; Wang, 2010]. Recent work in typically developing infants supports a role for gamma in early language acquisition. By 6 months of age, infants display increased gamma-band activity in response to native, but not non-native speech [Peña et al., 2010]. In addition, resting frontal gamma power (i.e., as collected using EEG without a particular time-locked task) has been associated with both receptive and expressive language ability [Benasich et al., 2008; Brito, Fifer, Myers, Elliott, & Noble, 2016; Gou et al., 2011; Tarullo et al., 2017]. Work by Benasich and colleagues has found that resting frontal gamma power is reduced in toddlers aged 24 and 36 months who have a family history of language impairment, and that gamma power is positively correlated with current language ability across a combined population of toddlers with and without family history of language impairment. Similarly, Tarullo and colleagues observed a positive relationship between resting frontal gamma in 4-year-old girls (but interestingly not boys) from rural Pakistan. However, in teenagers, resting gamma is negatively correlated with reading ability [Tierney, Strait, & Kraus, 2014], suggesting that the role of resting state gamma activity on language processes may depend on age. Many studies in older of children or adults with ASD have reported differences in gamma-band power compared to individuals without ASD; however, only a few studies have examined correlations with clinical symptoms [Cornew, Roberts, Blaskey, & Edgar, 2011; Maxwell et al., 2013; Orekhova et al., 2007; Rojas & Wilson, 2014], making it difficult to determine whether these differences are primary causes of impairments, or the result of ongoing compensatory mechanisms.

Accumulating research also suggests that there are significant neurobiological differences, including resting gamma power, in high-risk infant siblings (as compared to siblings of typically developing children) that are present well before symptom onset, and even among high-risk infants who do not later develop ASD [Elsabbagh & Johnson, 2010; Guiraud et al., 2011; Hazlett et al., 2017; Righi, Tierney, Tager-Flusberg, & Nelson, 2014; Riva et al., 2018; Seery, Vogel-Farley, Tager-Flusberg, & Nelson, 2013; Seery, Tager-Flusberg, & Nelson, 2014; Shen et al., 2017]. For example, our group reported that at both 3 and 6 months of age, high-risk infants, regardless of their later diagnosis, show reduced frontal EEG power across many frequencies [Levin, Varcin, O'Leary, Tager-Flusberg, & Nelson, 2017; Tierney et al., 2012]; however, these differences were reduced or no longer present by 24 months.

With regard to gamma oscillations, our lab using a subset of the data presented in this article, found differences between low- and high-risk groups in the baseline frontal gamma power developmental trajectory [Tierney et al., 2012]—the high-risk group had lower frontal gamma power at 6 months of age, but had similar gamma power by 24 months. This previous analysis, however, did not separate the high-risk group by ASD outcome, and did not correlate gamma power differences with concurrent or future language measures.

The current study aimed to investigate differences in baseline frontal gamma power and its association with language development in toddlers at high versus low familial risk for autism. First, using a substantially expanded data set (101 toddlers vs. 30 toddlers in our original study), we assessed whether baseline frontal gamma power at 24 months is altered between three outcome groups—low-risk without ASD (LR), high-risk without ASD (HR-NoASD), and high-risk with ASD (HR-ASD). Second, we assessed whether frontal gamma power at 24 months was associated with concurrent or future language ability, and whether these brain–behavior associations were different between outcome groups. Finally, given the mounting evidence that the pathophysiology and phenotype of ASD may be different between males and females, we investigated within-group differences between sexes and present data both combined and stratified by sex.

## Materials/Subjects and Methods

### *Participants*

Infants were enrolled in a longitudinal study of early neurocognitive development of infant siblings of children with ASD, conducted at Boston Children's Hospital/Harvard Medical School and Boston University. Institutional review board approval was obtained from both institutions (#X06-08-0374) prior to starting the study. Written, informed consent was obtained from all parents or guardians prior to their children's participation in the study.

All infants had a minimum gestational age of 36 weeks, no history of prenatal or postnatal medical or neurological problems, and no known genetic disorders (e.g., fragile-X, tuberous sclerosis). Furthermore, all infants were from primarily English-speaking households (English spoken more than 75% of the time). Infants designated as high-risk for ASD (HR) were defined by having at least one full sibling with a DSM-IV ASD diagnosis that could not be attributed to a known genetic disorder. All older siblings had a

community diagnosis of ASD, and in the majority of cases, this was confirmed using the Social Communication Questionnaire (SCQ) [Rutter Bailey & Lord, 2003] and/or the Autism Diagnostic Observation Schedule (ADOS) [Lord & Rutter, 2012].

Low-risk infants (LR) were defined by having a typically developing older sibling and no first- or second-degree family members with ASD. In the majority of cases, the siblings of LR infants were screened for ASD (67/72) using the SCQ, followed by the ADOS if concerns of ASD were raised.

A total of 255 participants were enrolled in the study. Given the longitudinal nature of the study and enrollment at an early age, 16 participants were excluded after enrollment as additional information was gathered and children no longer met our inclusion or exclusion criteria. In addition, three participants were excluded due to medical reasons that occurred during the study (hearing impairment, seizures, and new genetic finding).

Only a portion of the enrolled participants had high-quality EEG recorded at the 24-month time point, and was therefore included in this analysis. Three low-risk males went on to meet criteria for ASD and were not included in further analysis. Ultimately, 43 LR and 58 HR toddlers were included. 16/58 HR toddlers (27.6%) met criteria for ASD (Table 1).

*Behavioral Assessment*

Age-standardized T-scores from the Mullen Scales of Early Learning (MSEL) administered at 24 months of age by trained examiners were used to assessment development in four domains—receptive language, expressive language, fine motor, and visual reception. The ADOS was administered at 18, 24, and 36 months of age by research staff with extensive experience in testing children with developmental disorders, and then co-scored by an ADOS-reliable research assistant via video recording. For children meeting criteria on the ADOS, or coming within three points of cutoffs, a Licensed Clinical Psychologist reviewed scores and video recordings of concurrent and previous behavioral assessments, and using DSM-V criteria provided a best estimate clinical judgment: typically developing, ASD, or non-spectrum disorder (e.g., ADHD, anxiety, and language concerns). HR toddlers receiving a clinical judgment of either typically developing or non-spectrum disorders were classified as HR-NoASD, and those receiving a clinical judgment of ASD were classified as HR-ASD. Calibrated severity scores for the 24-month ADOS were determined to allow for comparison between individuals administered different ADOS modules.

*EEG Assessment*

Baseline EEG data were collected at 24 months of age in a dimly lit, sound-attenuated, electrically shielded room. The infant was held by their seated caregiver while a research assistant ensured the infant remained calm and still by blowing bubbles and/or showing toys. Caregivers were instructed by the research assistant to avoid social interactions or speaking with their child. Continuous EEG was recorded for 2–5 min. EEG data were collected using either a 64-channel Geodesic Sensor Net System or 128-channel Hydrocel Geodesic Sensor Nets (Electrical Geodesics, Inc., Eugene, OR) connected to a DC-coupled amplifier (Net Amps 200 or Net Amps 300, Electrical Geodesics Inc.). There was no difference in distribution of net type between outcome groups ($X^2_4 = 1.912$, $P = 0.38$).

**Table 1. Sample Characteristics**

| | LR $N = 43$ | HR-NoASD $N = 42$ | HR-ASD $N = 16$ | Fisher's exact test $P$ value |
|---|---|---|---|---|
| Sex | 24M, 19F | 20M, 22F | 11M, 5F | 0.36 |
| Maternal education, *n* (%) | | | | 0.01 |
| Not answered | 5 (11) | 1 (2) | 4 (25) | |
| <4-year college degree | 2 (5) | 9 (21) | 3 (19) | |
| 4-year college degree | 8 (19) | 7 (17) | 6 (37) | |
| Graduate degree | 28 (65) | 25 (60) | 3 (19) | |
| Paternal education, *n* (%) | | | | 0.17 |
| Not answered | 5 (11) | 1 (2) | 4 (25) | |
| <4-year college degree | 3 (7) | 3 (7) | 3 (19) | |
| 4-year college degree | 12 (28) | 15 (36) | 6 (37) | |
| Graduate degree | 23 (54) | 23 (55) | 3 (19) | |
| Household income, *n* (%) | | | | 0.67 |
| Not answered | 7 (16) | 2 (5) | 5 (31) | |
| <$75,000 | 5 (12) | 4 (9) | 2 (13) | |
| >$75,000 | 31 (72) | 36 (86) | 9 (56) | |
| Race, *n* (%) | | | | 0.07 |
| Non-white | 6 (14) | 2 (5) | 4 (25) | |
| Ethnicity, *n* (%) | | | | 0.25 |
| Hispanic or Latino | 2 (5) | 2 (5) | 1 (2) | |

Abbreviations: ASD, autism spectrum disorder; LR, low risk without ASD; HR-NoASD, high risk without ASD; HR-ASD, high risk with ASD.

Language scores also did not differ as a function of net type. Data were sampled at 250 or 500 Hz and referenced to a single vertex electrode (Cz), with impedances kept below 100 kΩ. Electrooculographic electrodes were removed to improve the child's comfort.

*EEG Preprocessing*

The continuous, non-task related EEG portion of the raw NetStation (EGI, Inc., Eugene, OR) files were exported to MATLAB (versionR2017a) for preprocessing and power analysis. All files were batch processed using the Batch EEG Automated Processing Platform [Levin, Méndez Leal, Gabard-Durnam, & O'Leary, 2018] to ensure uniform analysis regardless of when the EEG was acquired or which risk group they were in. A 1-Hz high-pass filter and 100 Hz low-pass filter were applied. Data sampled at 500 Hz were resampled using interpolation to 250 Hz. Both experimental and participant-induced artifacts were then identified and removed using the Harvard Automated Preprocessing Pipeline for EEG (HAPPE), a MATLAB based preprocessing pipeline optimized for developmental data with short recordings and/or high levels of artifact, to automate preprocessing and artifact removal, and to evaluate data quality in the processed EEGs [Gabard-Durnam, Mendez Leal, Wilkinson, & Levin, 2018]. While historically artifact removal has largely been accomplished through visual inspection, more recently the field has moved to more automated techniques that are less prone to human error and subjectivity and thus improve replicability, and allow for increased retention in data for analysis. HAPPE has been shown to both reject a greater proportion of artifact while simultaneously preserving underlying signal relative to manual editing. HAPPE also provides data output quality measures that can be used to systematically reject poor quality data unfit for further analyses. HAPPE artifact identification and removal include removing 60 Hz line noise, bad channel rejection, and participant produced artifact (eye blinks, movement, and muscle activity) through wavelet-enhanced independent component analysis (ICA) and multiple artifact rejection algorithm (MARA) [Winkler, Debener, Muller, & Tangermann, 2015; Winkler, Haufe, & Tangermann, 2011]. MARA was, in part, chosen for its excellent detection and removal of muscle artifact components, which can affect gamma signal [Gabard-Durnam et al., 2018; Winkler et al., 2011]. The following channels, in addition to the 10–20 electrodes, were used for MARA: 64-channel net—2, 3, 8, 9, 12, 16, 21, 25, 50, 53, 57, 58; 128-channel net—3, 4, 13, 19, 20, 23, 27, 28, 40, 41, 46, 47, 75, 98, 102, 103, 109, 112, 117, 118, 123. These electrodes focused on the frontal regions in order to improve our ability to detect and remove artifact in the region of our power analysis. After artifact removal using HAPPE, data were re-referenced to an average reference. Data were then detrended using the signal mean, and then regions of high-amplitude signal (>40 μV was used to account for the reduce signal amplitude post HAPPE processing) were removed prior to segmenting the remaining data into 2-sec windows to allow for power calculations using multitaper spectral analysis [Babadi & Brown, 2014]. Noncontinuous data were not concatenated. Additional analyses (before and after topoplots and spectral analyses) demonstrating the effectiveness removal of participant-induced artifact are provided in the Supporting Information.

*EEG Power Analysis*

A multitaper fast Fourier transform, using three orthogonal tapers [Thomson, 1982] was used to calculate a power spectrum on each segment for the following frontal electrodes: 64-channel net—2, 3, 8, 9, 12, 13, 58, 62.; 128-channel net—3, 4, 11, 19, 20, 23, 24, 27, 118, 123, 124 (Fig. S1). For each individual EEG and each electrode, the average power across all two-second segments was then calculated for the gamma band, defined as [30–50 Hz]. Gamma power was then averaged across electrodes for each individual to obtain their average frontal gamma power. Here we report absolute power values, normalized by a log 10 transform.

*EEG Rejection Criteria*

EEGs were rejected if they had fewer than 20 segments (40 sec), or were >3 standard deviations from the mean on the following HAPPE data quality output parameters: percent good channels (<82%), mean retained artifact probability (>0.3), median retained artifact probability (>0.35), percent of independent components rejected (>84%), and percent variance retained after artifact removal (<32%). Based on these criteria, 8 of the 148 EEGs collected at 24 months were rejected. Additionally, EEGs with a mean gamma power greater or less than 2 *SD* from their outcome group mean were reviewed blind to outcome group, leading to two additional EEGs to be rejected. Within the remaining data set, HAPPE data quality output parameters, including percent of independent components rejected (an indicator of the degree of artifact present prior to artifact removal) and the median and mean retained artifact probability (an indicator of the degree of artifact present in the processed EEG), were not significantly correlated with mean frontal gamma power (Pearson's $r$ values ranged from −0.16 to 0.1). In addition, mean frontal gamma power was not correlated with the amount of gamma power removed during our preprocessing steps (Pearson's $r = -0.029$, $P = 0.72$). These quality control measures increase our confidence that post-processed frontal gamma power measurements do

not reflect differences in initial, preprocessed, levels of artifact. We have also previously shown that the distribution of each of the above HAPPE data quality output parameters is similar across the three outcome groups [Gabard-Durnam et al., 2018].

### *Statistical Analyses*

A Fisher-exact test was used to characterize differences in demographic data between groups. All continuous variables within each outcome group were normally distributed using the Shapiro–Wilks test. Two-way ANOVA, followed by post hoc Bonferroni tests for multiple comparisons, were used to determine effects of group, sex, and group × sex interactions on head circumference, MSEL scores, ADOS calibrated severity scores, and frontal gamma power.

Logistic regression was used to determine whether frontal gamma power was associated with ASD diagnosis. Multivariate linear regression was used to characterize the relationship between frontal gamma power and MSEL language scores at 24 and 36 months. Multiple comparisons within models were adjusted for using False Discovery Rate.

All reported $P$ values are two-tailed, with a $P$ value of 0.05 indicating statistical significance. Analyses were performed using Stata software, version 14.2 (Stata). Figures were created using Python 2.7 and python data visualization libraries (*matplotlib* [Hunter, 2007] and *Seaborn* (https://seaborn.pydata.org/index.html)).

## Results

### *Sample Description*

The demographic data for each outcome group (LR, HR-NoASD, and HR-ASD) are provided in Table 1. There was a significant group difference in maternal, but not paternal education, with a higher proportion of mothers with less than a college degree in the HR-NoASD and HR-ASD groups compared to the LR group. There were no differences in household income, race, or ethnicity. Notably, the majority of participants were white with household income above $75,000.

### *Head Circumference*

Given recent reports of increased head circumference and early brain overgrowth in ASD populations, we examined whether there were differences in head circumference at 24 months within our sample population. There were no differences in head circumference between groups; however, there were expected differences between males and females, with females having smaller head sizes in all groups ($F(1,93) = 12.68$, $P = 0.0006$). There was no effect of group or group × sex interactions on head circumference (Table 2).

### *Group and Sex Differences in Developmental Profiles*

We next examined group (LR, HR-NoASD, and HR-ASD) and sex differences, as well as possible within-group sex differences on the MSEL subscales (expressive and receptive language, fine motor, and visual reception). Given differences in maternal education between groups, maternal education was included in the model as a covariate. There was a significant main effect of group on expressive language, and a significant interaction between effects of sex and group on receptive language (Table 2, Fig. 1). Specifically, HR-ASD toddlers had significantly lower MSEL expressive language T-scores compared to LR toddlers ($P = 0.02$, Bonferroni). For receptive language subscales, further post hoc analyses found significant group differences for females but not males ($P < 0.005$, Bonferroni), and that females in the HR-ASD group had lower receptive language T-scores compared to males ($P = 0.01$, Bonferroni). There were no effects of group or sex, or interaction effects of group and sex, on fine motor or visual reception measures.

Next, we examined sex differences in ASD symptoms at 24 months, using the ADOS calibrated severity score as the dependent variable and group, sex, and group × sex interactions as independent variables (Table 2). To control for possible confounding of language ability on ADOS severity, MSEL expressive and receptive language subscales were included as covariates. There was a significant interaction between the effects of sex and group. Post hoc analyses showed that both male and female HR-ASD toddlers had significantly increased severity scores compared to their respective counterparts in the LR group ($P = 0.006$; $P < 0.001$, Bonferroni). In addition, HR-ASD females had significantly increased severity scores compared to HR-NoASD females ($P < 0.001$); however, HR-ASD males had only marginally significant increased severity scores compared to HR-NoASDs males ($P = 0.06$). In line with this, HR-ASD females had significantly higher ADOS severity scores compared to HR-ASD males ($P = 0.005$).

Overall, in this study sample, high-risk females with ASD had the lowest expressive and receptive language scores, and highest ADOS severity scores. No differences between groups were observed for measures of fine motor and visual reception skills.

### *Frontal Gamma Power*

Next, we assessed group or sex differences in baseline frontal gamma power at 24 months of age. We hypothesized that HR-ASD toddlers would have significantly different frontal gamma power compared to LR and HR-NoASD toddlers. Two-way ANOVA was used to assess the effects of outcome group or sex, as well as possible group × sex interactions, on mean frontal gamma power. Given differences in head circumference between sexes,

**Table 2. 24-Month Sample Characteristics**

| | LR mean ± *SD* | | | HR-NoASD mean ± *SD* | | | HR-ASD mean ± *SD* | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Characteristics | Total | Male | Female | Total | Male | Female | Total | Male | Female | Group | Sex | Group × sex |
| *Head circumference*[a] | 49.3 ± 1.5 (*n* = *42*) | 50.0 ± 1.1 (*n* = *24*) | 48.4 ± 1.4 (*n* = *18*) | 49.2 ± 1.6 (*n* = *41*) | 49.8 ± 1.8 (*n* = *20*) | 48.5 ± 1.3 (*n* = *21*) | 49.4 ± 1.4 (*n* = *16*) | 49.6 ± 1.6 (*n* = *11*) | 49 ± 0.7 (*n* = *5*) | *df*(2,93) *F* = 0.06 0.946 | *df*(1,93) *F* = 12.68 0.0006 | *df*(2,93) *F* = 0.70 0.499 |
| *Mullen T-scores*[b] | *n* = 43 | *n* = 24 | *n* = 19 | *n* = 38–39 | *n* = 18–19 | *n* = 20–21 | *n* = 15 | *n* = 10 | *n* = 5 | *df*(2,78) | *df*(1,78) | *df*(2,78) |
| Expressive language | 60.2 ± 10.8 | 59.5 ± 10.8 | 61.0 ± 11.1 | 55.2 ± 8.8 | 54.1 ± 8.8 | 56.2 ± 8.9 | 46.1 ± 11.0 | 48.3 ± 10.6 | 41.6 ± 11.5 | *F* = 4.35 0.016 | *F* = 0.85 0.36 | *F* = 0.03 0.98 |
| Receptive language | 60.8 ± 7.8 | 59.7 ± 9.0 | 62.2 ± 6.0 | 56.2 ± 8.4 | 56.1 ± 6.4 | 56.3 ± 10.1 | 49.5 ± 13.4 | 55.8 ± 8.9 | 37 ± 12.5 | *F* = 10.75 0.0001 | *F* = 5.33 0.02 | *F* = 6.26 0.003 |
| Fine motor | 55.1 ± 10.6 | 54.1 ± 9.1 | 56.5 ± 12.3 | 53.5 ± 8.5 | 53.6 ± 7.6 | 53.3 ± 9.4 | 49.2 ± 10.6 | 53 ± 8.1 | 41.6 ± 11.8 | *F* = 1.31 0.28 | *F* = 0.47 0.49 | *F* = 1.06 0.35 |
| Visual reception | 59.9 ± 10.6 | 59.5 ± 9.5 | 60.5 ± 12.6 | 55.4 ± 9.9 | 54.9 ± 8.6 | 55.8 ± 11.1 | 53.2 ± 10.9 | 56.8 ± 10.3 | 46 ± 9.2 | *F* = 1.27 0.29 | *F* = 0.13 0.71 | *F* = 0.79 0.46 |
| *ADOS*[c] | *n* = 36 | *n* = 19 | *n* = 14 | *n* = 41 | *n* = 20 | *n* = 21 | *n* = 16 | *n* = 11 | *n* = 5 | | | |
| 24 months severity score | 1.63 ± 0.96 | 1.58 ± 0.9 | 1.29 ± 0.61 | 2.02 ± 1.25 | 2.35 ± 0.61 | 1.71 ± 0.78 | 4.56 ± 2.58 | 3.55 ± 2.11 | 6.8 ± 2.17 | *F* = 30.4 <0.0001 | *F* = 3.35 0.07 | *F* = 8.69 0.0004 |

Abbreviations: ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; HR-ASD, high risk with ASD; HR-NoASD, high risk without ASD; LR, low risk without ASD.
[a]Two-way ANOVA.
[b]Two-way ANOVA with maternal education as covariate.
[c]ANCOVA with MSEL expressive and receptive language T-scores as covariates.

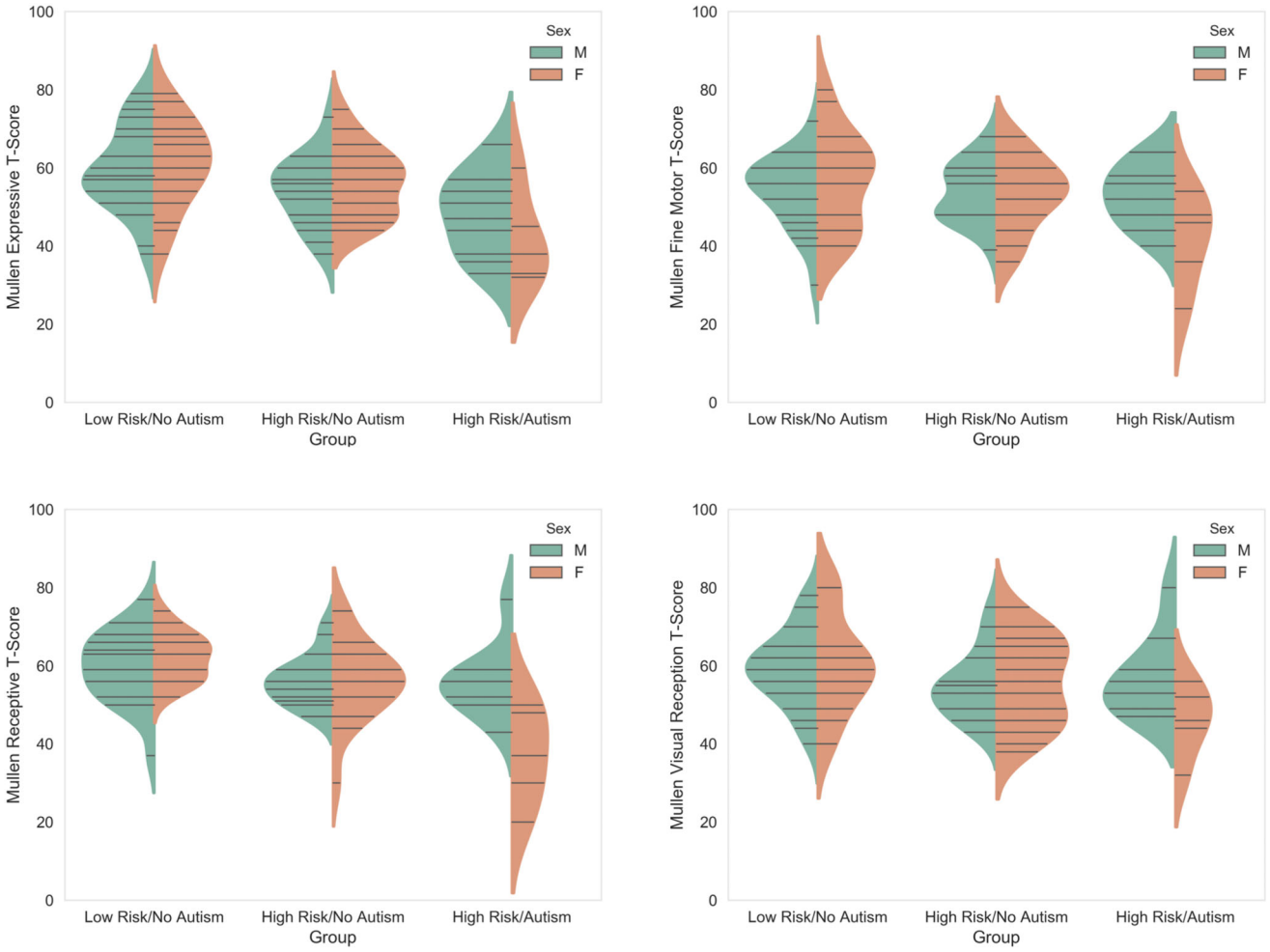


**Figure 1.** Mullen scales of early learning T-scores. Violin plots of T-scores from each of the four subscales (expressive language, receptive language, fine motor, and visual reception) are shown for each outcome group, divided into males and females. Lines represent individual data points. LR (*n*: males 24, females 19), HR-NoASD (*n*; males 18–19, females 20–21); and HR-ASD (*n*: males 10, females 5).

and differences in maternal education between groups, both were included as covariates.

A main effect of outcome group was present ($F_{2,80}$ = 4.73, $P$ = 0.01; Figure 2); however, contrary to our expectations, we found this was not due to HR-ASD differences, but rather reduced gamma power in the HR-NoASD group when compared to LR controls ($P$ = 0.013, Bonferroni). There was no difference between males and females, and no significant group × sex interactions.

This finding suggests that *within* a high-risk population, increased frontal gamma at 24 months of age may be associated with ASD diagnosis. However, within the high-risk population frontal gamma power was only marginally associated with ASD diagnosis in a logistic regression model that adjusted for sex and maternal education (odds ratio per 1-*SD* increase in frontal gamma power, 2.1; 95% CI, 0.98–4.6, $P$ = 0.06). In addition, this association was further reduced when MSEL verbal quotient was added as a covariate (odds ratio, 1.5, 95% CI, 0.6–3.48, $P$ = 0.4), emphasizing the strong relationship between ASD diagnosis and language.

### *Frontal Gamma and Concurrent MSEL Language Scores*

The close relationship between language and ASD outcome creates challenges in identifying neural correlates that are specific to ASD. Do aberrant gamma measurements in ASD populations represent brain changes that are specific to ASD, or do they represent highly associated developmental phenotypes, such as language delay or cognitive challenges, that are not core features of ASD? In the present study's sample population, reduced frontal power across multiple frequency bands was observed at 3 months of age in the high-risk group, well before ASD symptoms are present [Levin et al., 2017], and remain reduced in the HR-NoASD, but not the HR-ASD group at 24 months of age. This suggests that atypical gamma power may not be specific to ASD outcome, but a broader

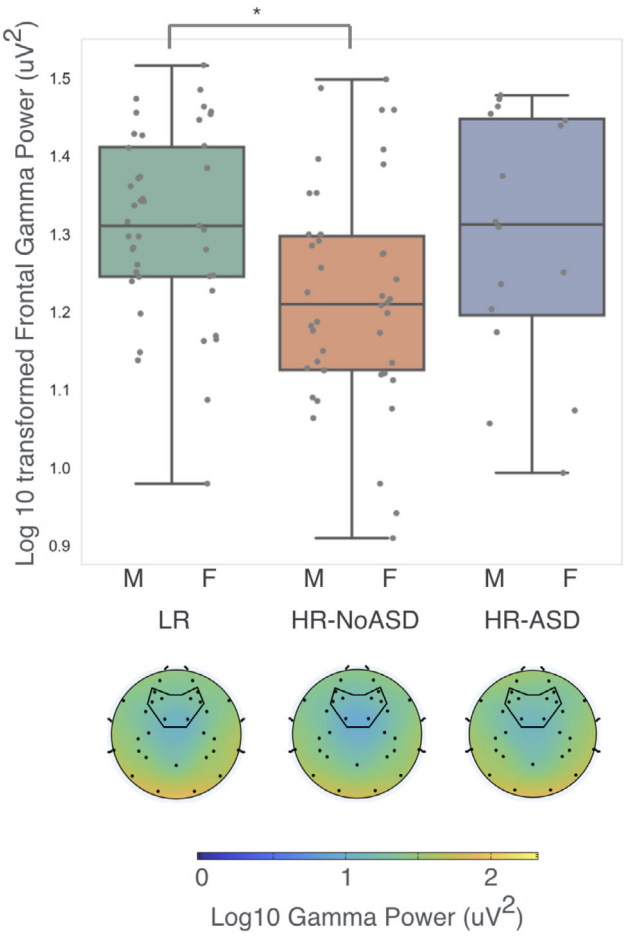


**Figure 2.** Frontal gamma power reduced in HR-NoASD group. Box plots of frontal gamma power are shown for each outcome group. Individual data points for males (left) and females (right) are also shown for each group. Mean values: LR ($n$ = 43, 1.31 ± 0.12); HR-NoASD ($n$ = 42, 1.22 ± 0.14); and HR-ASD ($n$ = 16, 1.30 ± 0.16). Two-way ANOVA test, controlling for head circumference and maternal education, showed main effect of group ($F_{2,80}$ = 4.73, $P$ = 0.01) with reduced frontal gamma in HR-NoASD group compared to LR group (Bonferroni, $P$ = 0.013). Topoplots of gamma power are also shown for each group with the electrodes used for frontal power calculations outlined.

developmental process. To investigate this further, we next asked whether the relationships between frontal gamma power and MSEL language scores are different between risk and outcome groups.

Initially, using simple, unadjusted, Pearson correlations (Fig. 3) between risk groups, we found in high-risk toddlers that frontal gamma power was negatively correlated with MSEL expressive ($r$ = −0.24, $P$ = 0.01, $n$ = 54), but not receptive subscales ($r$ = −0.2, $P$ = 0.15, $n$ = 54). No correlation between gamma and language scores was observed in low-risk toddlers (expressive: $r$ = 0.01, $P$ = 0.94, receptive: $r$ = 0.04, $P$ = 0.8; $n$ = 43). When the high-risk group was divided into outcome groups, this negative correlation between frontal gamma and expressive language was maintained in the HR-NoASD group ($r$ = −0.31, $P$ = 0.05, $n$ = 39). A similar, but not significant trend was observed in the HR-ASD group ($r$ = −0.21).

To further evaluate the effect of risk and outcome group on the relationship between frontal power and expressive language, and to describe any within-group differences between sexes, two linear regression models were further examined, using MSEL expressive subscales as the dependent variable. Model 1 (adjusted $R^2$ = 0.16) included both two-way and three-way interactions between risk (low vs. high risk), sex, and frontal gamma. Model 2 (adjusted $R^2$ = 0.17) included both two-way and three-way interactions between outcome group (LR, HR-NoASD, and HR-ASD), sex, and frontal gamma. Three-way interactions for both models had $P$ values less than 0.25 and were therefore retained (Table 3). Both models also included head circumference and maternal education as covariates. In order to specifically evaluate the relationship between MSEL expressive language subscales and frontal gamma power within risk or outcome subgroups, a marginal effects analysis was conducted and slopes are presented in Table 3.

***Model 1.*** Slope comparisons of subgroups from Model 1 revealed that high-risk toddlers showed a significant negative effect of frontal gamma power on expressive language (unadjusted $P$ = 0.007; adjusted $P$ = 0.014), while low-risk toddlers did not. However, the effect of frontal gamma power on expressive language scores was not significantly different between risk groups. Within risk groups, there was no significant difference between males and females.

***Model 2.*** Slopes of MSEL expressive language T-scores versus frontal gamma power from Model 2 are also shown in Table 3. However, given the small number of participants in HR-ASD group, these results should be interpreted with caution. Between outcome groups, HR-ASD toddlers had the strongest negative association (unadjusted $P$ = 0.04, adjusted $P$ = 0.12). However, the effect was not significantly different from LR or HR-NoASD groups. When groups were further subdivided by sex, the strongest negative relationship between frontal gamma and expressive language was observed in HR-NoASD females and HR-ASD males (Table 3, see Fig. S2 for scatterplots).

### *Frontal Gamma and Future MSEL Language Scores*

Finally, we assessed associations between frontal gamma power at 24 months and later language ability at 36 months (Table 3). In this third model (adjusted $R^2$ = 0.19), LR and HR-ASD groups significantly differed in their associations ($F_{1,53}$ = 4.36, $P$ = 0.04), with the LR group having a

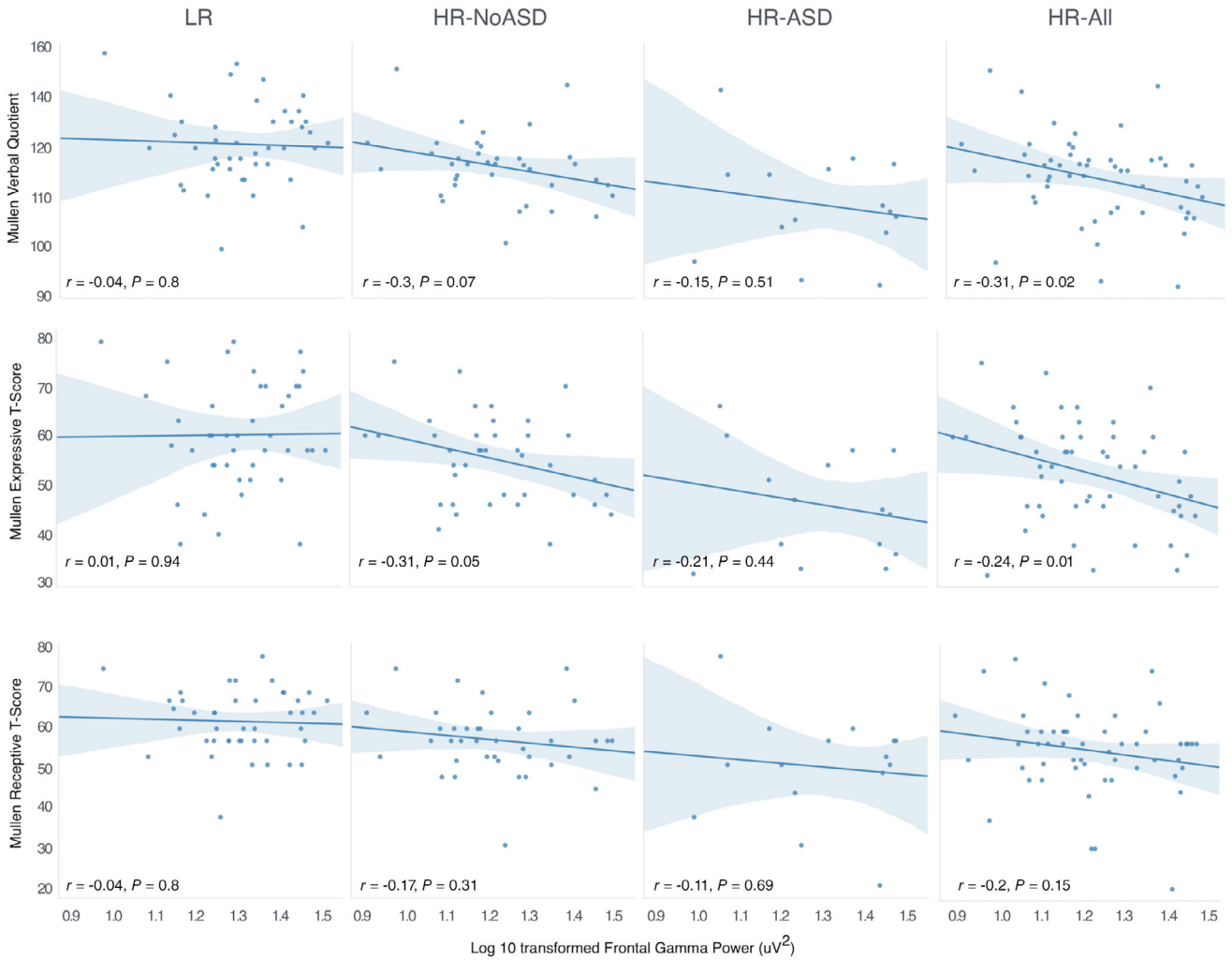


**Figure 3.** Frontal gamma power and Mullen scales of early learning language scores. Frontal gamma is negatively correlated with the Mullen verbal quotient score for high-risk toddlers (HR-ALL), but not low-risk toddlers (LR). When divided into language subscales, this negative correlation was only significant for expressive, but not receptive language T-scores. When further divided into outcome groups, only high-risk toddlers without autism (HR-NoASD) showed significant negative correlation between frontal gamma and expressive language T-scores.

positive association, and the HR-ASD having a negative association. When groups were subdivided by sex, a stronger positive association between frontal gamma power and later language scores was observed in LR females compared to LR males.

## Discussion

Here we report that at 24 months of age, resting frontal gamma power was significantly reduced in high-risk toddlers *without* ASD compared to low-risk controls; however, no difference was observed between high-risk toddlers *with* ASD and low-risk controls, suggesting that the single measure of resting gamma power is not a useful biomarker of ASD—at least at 24 months. Furthermore, higher gamma power in the high-risk group was marginally associated with ASD outcome ($P$ = 0.06), and this association was not maintained when language ability was added as a covariate, emphasizing the strong linkage between ASD diagnosis and language skills.

### *Gamma and Language*

Our lab's previous longitudinal analysis from a smaller subset of this study population found that high-risk infants (collapsed across ASD outcome) at 6 months had lower power across all frequency bands, but by 24 months gamma power was similar between high- and low-risk infants [Tierney et al., 2012]. Our current finding that HR-NoASD toddlers have reduced frontal gamma power at 24 months was unexpected. Further subgroup analysis of the original 30 participants included in the Tierney et al. [2012] paper showed a similar trend to our current results, with the HR-NoASD group (mean = $1.22 \pm 0.13$; $n$ = 16) having reduced frontal gamma power compared with both

**Table 3. Effect of 24 months Frontal Gamma Power on MSEL Expressive T-Score[a]**

| Model 3-way interactions | | *P* value |
|---|---|---|
| Model 1 | Sex × risk × gamma | 0.137 |
| Model 2 | Sex × HR-neg × gamma | 0.08 |
| | Sex × HR-ASD × gamma | 0.414 |
| Model 3 | Sex × HR-neg × gamma | 0.22 |
| | Sex × HR-ASD × gamma | 0.68 |

| MSEL expressive language T-score versus frontal gamma | | | |
|---|---|---|---|
| | Slope (95% CI) | Unadjusted *P* value | Adjusted *P* value |
| **Model 1** | | | |
| Low risk | −13.3 (−42.1 to 15.4) | 0.359 | 0.359 |
| Males | −35.2 (−81.4 to 11.0) | 0.13 | 0.17 |
| Females | 12.4 (−19.4 to 44.2) | 0.43 | 0.43 |
| High risk | −25.5 (−43.9 to −7.0) | 0.007 | 0.014 |
| Males | −24.1 (−51.5 to 3.2) | 0.08 | 0.160 |
| Females | −27.1 (−50.9 to −3.3) | 0.03 | 0.12 |
| **Model 2** | | | |
| LR | −13.3 (−42.3 to 15.7) | 0.36 | 0.36 |
| Males | −34.4 (−81.0 to 12.3) | 0.15 | 0.30 |
| Females | 11.4 (−20.7 to 43.6) | 0.48 | 0.576 |
| HR-NoASD | −14.3 (−37.4 to 8.7) | 0.22 | 0.33 |
| Males | −4.5 (−43.3 to 34.3) | 0.82 | 0.82 |
| Females | −23.6 (−49.9 to 2.7) | 0.08 | 0.24 |
| HR-ASD | −40.1 (−77.6 to −2.6) | 0.04 | 0.12 |
| Males | −41.2 (−87.7 to 5.2) | 0.08 | 0.24 |
| Females | −37.1 (−103.7 to 29.4) | 0.27 | 0.41 |
| **Model 3 (36 month expressive T-score)** | | | |
| LR | 15.8 (−15.4 to 47.1) | 0.31 | 0.47 |
| Males | −4.4 (−52.3 to 43.6) | 0.86 | 0.86 |
| Females | 40.7 (6.3 to 75.0) | 0.02 | 0.12 |
| HR-NoASD | 6.3 (−16.8 to 29.4) | 0.59 | 0.59 |
| Males | 6.2 (−28.9 to 41.2) | 0.73 | 0.86 |
| Females | 6.5 (−22.0 to 35.0) | 0.65 | 0.86 |
| HR-ASD | −33.1 (−70.0 to 0.8) | 0.06 | 0.18 |
| Males | −42.5 (−88.0 to 3.0) | 0.07 | 0.21 |
| Females | −17.3 (−74.4 to 39.7) | 0.54 | 0.86 |

[a]Results above are from linear regression models in which the outcome variable was MSEL expressive language T-scores. The independent variables were frontal gamma power, sex, and risk (Model 1) or group (Models 2 and 3). Full factor interactions of independent variables were included in the models. Potential confounders, head circumference and maternal education, were included as covariates. Slopes presented are for frontal gamma power and MSEL expressive T-score. Both unadjusted and adjusted *P* values for multiple comparisons, using False Discovery Rate are presented.

the LR-NoASD group (mean = 1.32 ± 0.14; $n = 10$) and the HR-ASD group (mean = 1.34 ± 0.19, $n = 4$), although given the reduced $n$, these differences were not significant (ANOVA $F_{2,27} = 2.09$, $P = 0.14$).

One possible hypothesis based on these findings is that *maintenance* of reduced frontal gamma across the first 2 years may be a marker of improved developmental outcome. In support of this hypothesis, low frontal gamma power was associated with better language ability in the high-risk toddlers. However, there was no such association in the low-risk group. Interestingly, in a similar age group, Benasich et al. have reported reduced frontal gamma in toddlers with familial risk for language impairment. However, they did not evaluate the association between gamma and language function within this subset of children, rather they found gamma to be positively correlated with language *across* a larger sample, which combined participants both with and without familial risk of language impairment [Benasich et al., 2008; Gou et al., 2011]. In our study, we only observed this positive relationship in LR females when comparing frontal gamma power at 24 months to expressive language scores at 36 months. While Benasich et al. had similar numbers of males and females in their enrolled population, only a subset had EEG and behavioral data, and the breakdown of males versus females for each age group analyzed was not reported. A study of over 100 4-year-olds from rural Pakistan also found gender differences in the frontal gamma-language relationship, with a significant positive association observed only in females [Tarullo et al., 2017]. Together, our data suggest that sex may play an important role in this relationship.

Why would reduced gamma in a high-risk population be associated with improved language ability? Gamma activity is associated with a variety of higher order cognitive processes including language [Mcfadden, Hepburn, Winterrowd, Schmidt, & Rojas, 2012; Peña et al., 2010], attention [Fries, Nikolić, & Singer, 2007; Taylor, Mandon, Freiwald, & Kreiter, 2005], and working memory [Howard et al., 2003; Pesaran, Pezaris, Sahani, Mitra, & Andersen, 2002]. However, gamma oscillations also indirectly represent the balance between excitatory and inhibitory neurons. Gamma oscillations in the cortex are generated by parvalbumin (PV) inhibitory interneurons; however, disruption in PV interneurons in rodents has been shown to both increase and decrease spontaneous gamma power [Sohal, 2012]. Reduced gamma oscillations in the context of aberrant neurocircuitry present earlier in development may represent a variety of functions including successful compensation for processes that may increase gamma oscillations such as PV hypofunction. Alternatively, increased gamma in already abnormal neurocircuitry may lead to a ceiling effect, preventing further increase in gamma during cognitive processes. In this case, reduced gamma power would provide a more pliable system for learning. Teasing this out further is a challenging task. Longitudinal analysis of baseline gamma focused on differences between both group, and sex within group, will be useful. In addition, future studies evaluating the relationship between baseline gamma and evoked gamma within outcome groups, and how this relates to language will improve our understanding of the developmental role of gamma activity within high-risk populations.

There is also debate on what gamma power measured by scalp EEG represents, as studies have shown contamination in the gamma band by non-EEG signals, such as

myogenic or ocular artifacts, and there is concern that artifact cannot be fully removed [Goncharova, McFarland, Vaughan, & Wolpaw, 2003; Muthukumaraswamy, 2013]. The HAPPE automated pipeline used in this analysis utilizes a combination of ICA decomposition, wavelet-enhanced ICA, the ICA extended-Infomax algorithm, MARA, and segment rejection, and has been demonstrated to remove 85%–90% of artifact prior to the last segment rejection step. Supporting Information in this article shows pre- and post-artifact removed topoplots and power spectra from individuals. While it is likely that some artifact remains within the gamma band, it is unlikely that remaining artifact alone is driving the central findings of this article, as no differences were observed between groups in HAPPE data quality measures, and there was no relationship between various measures of initial artifact (percent IC's removed; frontal gamma power removed by preprocessing) and post-processed gamma power.

*Sex Differences*

Given the growing evidence of sex differences in early brain development and plasticity in ASD [Baron-Cohen Simon, 2010; Kim et al., 2013; Lai et al., 2017; Mottron et al., 2015; Werling, 2016], in addition to differences in prevalence and phenotype between sexes, this study closely examined any possible within-group sex differences. Prospective studies of familial high-risk infants provide a unique opportunity to investigate possible compensatory mechanisms that "protect" females from ASD. Given our limited sample size, strong conclusions cannot be made with regard to sex differences. However, evaluating data sub-grouped by sex is important for building hypotheses for future studies. In this study, female high-risk toddlers with ASD ($n$ = 5) had significantly lower receptive language skills than their male counterparts, and increased ADOS severity scores. Reduced IQ in females with ASD has been observed by several other groups [Lord, Schopler, & Revicki, 1982; Volkmar, Szatmari, & Sparrow, 1993], however others, specifically investigating high-risk infants in a larger sample size than this study, did not observe within-group sex differences in cognitive functioning or ASD symptoms severity [Messinger et al., 2015]. In this study, there were no significant differences between sexes across outcome groups in frontal gamma power at 24 months. However, when individual data points are examined, high-risk females make up a larger proportion of the lowest quartile of mean frontal gamma power (Fig. 2). Furthermore, we observed that HR-NoASD females and HR-ASD males have the strongest negative relationship between frontal gamma power and language ability. One possible explanation for this similarity is that these two subgroups have the greatest similarities in underlying neurobiology. While few studies have focused on genetic risk factors in *unaffected* high-risk females, the increased genetic burden observed in females with ASD suggests that at least a portion of unaffected high-risk females have a genetic burden similar to that seen in affected males.

*Limitations*

This study has several limitations. Given the longitudinal nature of the study, EEG acquisition changed over the course of the study. Two types of nets were used and EEGs were collected at two sampling rates. Given this variation, we utilized batch preprocessing methods and artifact removal specific for infant EEG data to reduce any additional differences in data analysis. In addition, analyzed electrodes for each net type were carefully selected using EGI published reports [Luu & Ferree, 2005] to ensure the same regions of interest were represented for each net type. A second limitation is that while this was a large study, enrolling over 100 HR infants, our sample size of HR-ASD toddlers with high-quality EEG data at 24 months was small ($n$ = 16), limiting our statistical power within this group. Third, our participants, including those diagnosed with ASD, generally had age-appropriate language abilities. Limited variability of language skills within groups may have hindered our ability to observe statistically significant associations. Fourth, due to the young age of the children, attentional state and behaviors cannot be fully controlled. Our acquisition paradigm was designed to maximize recording duration (2–5 min) with the expectation that there would be some variability in behaviors and emotional states during acquisition, and power would be averaged across a large number of 2-sec epochs. While an observer was present in the room to monitor and manage behaviors and attention, we cannot fully rule out that group differences in EEG power are related to group differences in toddler state or behavior during acquisition. For example, social stimulation and exploratory behavior increase theta power, and attentional state alters alpha power [Jones et al., 2015; Orekhova, Stroganova, Posikera, & Elam, 2006]. While differences in behavioral state have not been shown to affect gamma power, it is possible that the reduced gamma power observed in the HR-NoASD group reflects a difference in behavioral state that is also reflective of language ability.

A larger goal of this study was to investigate the utility of EEG as a clinical biomarker of language development in high-risk infants. The utility of EEG as a biomarker is dependent on its ability to predict risk, outcome, or treatment response, and does not rely on fully understanding the neural and non-neural contributions of the measure, although such understanding would guide the development of therapeutic interventions. Biomarkers must also be easily reproducible in the often less constrained clinical environment. While our findings alone do not yet present sufficient evidence for the use of EEG frontal gamma power as a

biomarker of language development, we have used simple EEG acquisition parameters and a freely available automated preprocessing pipeline in order to encourage replication and reproducibility on both a research and clinical scale.

*Conclusions*

We found that high-risk toddlers *without* ASD have reduced baseline frontal gamma activity, and that within this study's high-risk population low frontal gamma power was associated with better language ability. Furthermore, this negative association between gamma power and language was largely driven by the high-risk females, emphasizing the importance of sex subgroup analysis. Together these findings suggest that gamma assessed at this age may represent the result of ongoing compensatory mechanisms. To better understand the role of measured gamma activity in ASD, we must disentangle longitudinal compensatory changes in neural circuitry from core features of brain dysfunction. This requires both longitudinal analysis of high-risk populations, starting very early in life, as well as continued investigation into the relationship between baseline and evoked gamma power throughout the course of early development.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Author Contributions

CLW was involved in study conception, performed the EEG and behavioral data analysis, interpreted the data, and drafted the manuscript. ARL and LJGD contributed to EEG preparation and analysis, and critically revised the manuscript for intellectual content. HTF and CAN were responsible for the study design, overseeing data acquisition, and critically reviewed the manuscript for intellectual content.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Supplementary Figure 1.** Electrodes used for power analysis are outline in red for 64-channel net (left) and 128-channel net (right)

**Supplementary Figure 2.** Frontal Gamma Power and MSEL Expressive Language Scores by outcome group and sex

**Supplementary Figure 3.** Averaged topographic distribution plots across three outcome groups (LR-NoASD, HR-NoASD, and HR-ASD), for frequency bands Delta (2-4 Hz), Theta (4-6 Hz), Low Alpha (6-9 Hz), High Alpha (9-13 Hz), Beta (13-30 Hz) and Gamma (30-50 Hz), after minimal artifact removal or after HAPPE/BEAPP artifact removal outlined in methods section. Electrodes used for topoplots were limited to those included in our HAPPE ICA decomposition *and* present on both 64 and 128 channel nets. These electrodes are shown on the Gamma topoplots with red electrodes representing those exclusively used in the frontal gamma power analysis performed in this paper. On the right, averaged power spectra (after preprocessing) are shown for 10 electrodes across the scalp. Note the prominent mu rhythm (7-9 Hz), associated with "behavioral stillness", in the C3 and C4 electrodes in all groups. As expected, artifact removal from frontal regions was better than occipital regions where fewer electrodes were used for ICA decomposition.

**Supplementary Table 1.** HAPPE data quality measures averaged by group and for individuals presented in Supplementary Figure 4.