Published in final edited form as:

J Autism Dev Disord. 2015 January ; 45(1): 4–14. doi:10.1007/s10803-013-1820-0.

Maternal Gesture Use and Language Development in Infant Siblings of Children with Autism Spectrum Disorder

Meagan R. Talbott and Dr. Helen Tager-Flusberg

Psychology Department at Boston University, Boston, MA, USA

Abstract

Impairments in language and communication are an early-appearing feature of autism spectrum disorders (ASD), with delays in language and gesture evident as early as the first year of life. Research with typically developing populations highlights the importance of both infant and maternal gesture use in infants' early language development. The current study explores the gesture production of infants at risk for autism and their mothers at 12 months of age, and the association between these early maternal and infant gestures and between these early gestures and infants' language at 18 months. Gestures were scored from both a caregiver-infant interaction (both infants and mothers) and from a semi-structured task (infants only). Mothers of non-diagnosed high risk infant siblings gestured more frequently than mothers of low risk infants. Infant and maternal gesture use at 12 months was associated with infants' language scores at 18 months in both low risk and non-diagnosed high risk infants. These results demonstrate the impact of risk status on maternal behavior and the importance of considering the role of social and contextual factors on the language development of infants at risk for autism. Results from the subset of infants who meet preliminary criteria for ASD are also discussed.

Keywords

gesture; language; infant siblings; autism

Impairments in language and communication are core features of autism spectrum disorders (ASD), ranging from the pragmatic deficits observed in higher-functioning individuals to the often profound delays exhibited by those more severely affected (Tager-Flusberg, Paul, & Lord, 2005). These impairments are also among the earliest emerging behavioral markers of ASD, with deficits in language and gesture production identified retrospectively as early as 12 months of age (Colgan et al., 2006; Osterling and Dawson, 1994; Werner, Dawson, Osterling, & Dinno, 2000). Prospective investigations of infant siblings of children with autism, who are at an increased genetic risk for the disorder (see Zwaigenbaum, et al., 2007 for a discussion of this methodology) have also reported delays in language and gesture production during the first year of life for infants who are later diagnosed (Mitchell, et al., 2006; Ozonoff, et al., 2010; Paul, Fuerst, Ramsay, Chawarska, & Klin, 2011; Rozga et al., 2010; Zwaigenbaum, Bryson, Rogers, Roberts, Brian, & Szatmari, 2005). Importantly,

prospective investigations of these high risk infants (HRA), defined by having an older sibling with an ASD diagnosis, have also identified impairments in language and gesture use in non-diagnosed infant siblings, although these impairments are typically reported beginning in the second year of life (Ben-Yizhak, Yirmiya, Seidman, Alon, Lord, & Sigman, 2011; Gamliel, Yirmiya, Jaffe, Manor, & Sigman, 2009; Iverson & Wozniak, 2007; Mitchell et al., 2006; Stone, McMahon, Yoder, and Walden, 2007; Yirmiya, Gamliel, Pilowsky, Feldman, Baron-Cohen, & Sigman, 2006; Yirmiya, Gamliel, Shaked, & Sigman, 2007; Zwaigenbaum et al., 2005). Given the pervasiveness of language difficulties within the entire group of high risk siblings and the impact of language on functional outcomes, understanding the factors that underlie early language development within this population has significant implications not only for the subset who are diagnosed with ASD, but for a significant portion of siblings whose diagnostic status may be less clear.

A variety of early developmental domains have been identified as correlates of language ability in young children with ASD, including early motor development (Iverson & Wozniak, 2007), cognition (Thurm, Lord, Lee, Newschaffer, 2007), play, (Sigman & McGovern, 2005), imitation (Charman, Baron-Cohen, Swettenham, Baird, Drew, & Cox, 2003; Toth, Munson, Melzoff, & Dawson, 2006), joint attention (Adamson, Bakeman, Deckner, & Ronski, 2009; Mundy, Sigman, & Kasari, 2000; Shumway & Wetherby, 2009) and gesture use (Luyster, Kadlec, Carter, & Tager-Flusberg, 2008; Smith, Mirenda, & Zaidman-Zait, 2007).

In a study examining the contribution of each of these domains to the language level of toddlers with ASD, cognitive level and gesture use were identified as the most robust predictors of both receptive and expressive concurrent language levels (Luyster et al., 2008). Given the considerable overlap between early gesture production and the pre-linguistic communicative functions they serve, including many of the specific domains mentioned above (e.g. initiation of joint attention) it is not surprising that gesture use accounted for a significant proportion of variance in the models for both language domains.

The impact of gesture use on early language development is underscored by work with typically developing infants, in which specific features of gesture production, such as the diversity of meanings they express, and their combination with infants' early vocabulary (e.g. pointing to juice while saying 'more'), predict specific measures of language development, namely vocabulary size and sentence complexity (Iverson & Goldin-Meadow, 2005; Rowe, & Goldin-Meadow, 2009a).

Several investigations have also reported relationships between early maternal gesture use and infants' own gesture use and language ability. Tomasello and Farrar (1986) found that maternal directives (orienting the child's attention to a new object through speech) which included gestures and which the infant followed to the target, were directly correlated with infants' concurrent vocabulary at 15 months. Iverson, Capirci, Longobardi, and Caselli (1999) also reported significant correlations between maternal gesture and infant gesture and language measures, although they dropped below significance when controlling for overall maternal communicativeness. Finally, maternal gesture use predicts concurrent infant gesture use, which in turn predicts infants' language development, in some cases more than

2 years later (Liszkowski, Brown, Callaghan, Takada, de Vos, 2012; Liszkowski & Tomasello, 2011; Rowe & Goldin-Meadow 2009a; Rowe, Özçaliskan & Goldin-Meadow, 2008).

The relationship between maternal gestures and infant language development is of particular interest for infants at risk for ASD given the difficulties infants and children with ASD demonstrate in social communication, including gaze, social orienting, and attention shifting (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Landry and Bryson, 2004; Nadig, Ozonoff, Young, Rozga, Sigman, & Rogers, 2007; Ozonoff et al., 2010, Zwaigenbaum, et al., 2005). There is evidence that these domains may also be vulnerable in non-diagnosed infant siblings. Six-month-old HRA infants display atypical patterns of social attention, spending more time looking away from their parents during a Face-to-Face/Still-Face Paradigm than a group of age-matched controls (Ibanez, Messinger, Newell, Lambert, & Sheskin, 2008). 12-to-23-month old HRA siblings demonstrate impairments in directing attention, and are also less accurate at following an experimenter's cues to a target, although improve when the experimenter's cues are highly redundant and include gaze shifts, vocalizations, and pointing (Presmanes, Walden, Stone, and Yoder; 2007; Stone et al., 2007). These impairments may limit HRA infants' use of maternal gestures when acquiring language.

There is also reason to suspect that maternal gesture production itself may be altered in mothers of high risk infants. Recent work with typically developing mother-infant dyads demonstrates the importance of reciprocity in early gestural 'proto-conversations', with both mothers and infants more likely to point within close temporal proximity of each other's point production (Liszkowski et al., 2012). Although it is not yet clear whether impairments in gesture production are present in non-diagnosed high risk siblings at 12 months of age, the presence of such delays may result in fewer reciprocal gestural interactions, and thus lead to corresponding reductions in maternal gesture production rates. Mothers of high risk infants may also differ in the distribution of the types of gestures they produce. Such alterations in gesture distribution have been identified in other clinical populations such as Down Syndrome, where mothers produce a greater proportion of deictic gestures than mothers of typically developing infants matched on language level; this increase in deictic gestures is part of a pattern of 'gestural motherese' which functions to simplify maternal communication (Iverson, Longobardi, Spampinato, & Caselli; 2006).

Although only a subset of high risk infants will go on to develop ASD, mothers of high risk infants may use some of the adaptations they have learned with their older diagnosed child, including increased gesture production, during interactions with their high risk infants. There is emerging evidence to support this claim. Beginning as early as 6 months, mothers of high risk infants are rated as more directive during parent-child interactions and also report significantly more concerns than parents of low risk infants (McMahon, Malesa, Yoder, and Stone, 2007; Ozonoff et al., 2009, Wan, et al., 2012). Importantly, while parental concerns in the second year of life are well correlated with infants' diagnostic status, these very early concerns are poorly related to standardized measures of concurrent infant functioning, suggesting that alterations in parent behavior at the earliest ages are more likely a function of risk status in general, rather than a specific reflection of symptom emergence

(Ozonoff et al., 2009). These early concerns, combined with parents' experiences with their older diagnosed child, may manifest in parents' adoption of a gestural motherese, including increased use of deictic gestures, during early mother-child interactions.

In sum, although the literature has identified delays in gesture use by both diagnosed and non-diagnosed high risk infant siblings, it is unclear whether to expect mothers of high risk infants to demonstrate differences in gesture production at 12 months of age and whether any potential differences in maternal gesture use would lead to diminished, enhanced, or preserved effects of maternal gesture on the gesture and language development of high risk infants.

Here, we pursue three particular goals. The first is to examine the potential impact of risk status on parental gesture use by comparing both the quantity and diversity of gestures used by mothers when interacting with their 12-month-old infants. The second is to determine whether delays in gesture are observed in HRA infants at 12 months of age. Finally, we hope to determine whether the relationships between maternal gesture, infant gesture, and infant language development observed in the typically developing literature are altered in this high risk population.

Methods

Participants

Data for the 75 infants (48 HRA, 27 LRC) included in this study were obtained as part of a larger ongoing study of infants at risk for ASD conducted jointly by Boston University and Boston Children's Hospital. Interested families were contacted by the study coordinator, who conducted a detailed telephone eligibility interview. All infants were screened for exclusionary criteria (prematurity, extended stays in the neonatal intensive care unit, maternal drug or alcohol use during pregnancy, family history of genetic disorders associated with ASD, and primary languages other than English). Infants were enrolled into the high-risk group (HRA) if they had an older sibling with a diagnosis of Autism, Asperger Syndrome, or Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS); diagnostic information for the proband was obtained through parent report during the telephone screening process and confirmed for probands at least 48 months of age by a score of at least 15 on the Social Communication Questionnaire (SCQ; Rutter, Bailey, & Lord, 2003; $n = 29$), meeting diagnostic criteria on the Autism Diagnostic Observation Schedule – Revised (ADOS; LORD et al., 2000; $n = 6$), or through diagnosis by expert clinician in the community ($n = 12$). Infants were enrolled into the low risk control (LRC) group if they had at least one older sibling who was typically developing, confirmed by a score less than 12 on the SCQ for those over 48 months of age, and no first-degree relatives diagnosed with an ASD or other neurodevelopmental disorder. There were no group differences on any of the demographic variables, which are presented in Table 1. Informed consent was obtained from parents prior to participation. [Insert table 1 about here]

Procedure

Infants were seen at 12 and 18 months as part of their participation in the larger ongoing project. At the 12-month visit, trained research staff administered the Communication and Symbolic Behavior Scale (CSBS; Wetherby, & Prizant; 1993), the Mullen Scales of Early Learning (MSEL; Mullen, 1995), and infants and parents engaged in a 10-minute free play session (mother-child interaction; MC) during which they were provided with a variety of infant-appropriate toys (rattles, book, cars, etc.) and were instructed only to “play as you normally would”. For a small number of infants (3 HRA, 1 LRC) fathers served as the primary caregiver and were included in these analyses. For ease of interpretation, all interactions are referred to as mother-child dyads. At the 18-month visit, trained research staff administered the MSEL and the Autism Diagnostic Observation Schedule.¹ All sessions were video recorded for later coding and transcription.

Measures

Communication and Symbolic Behavior Scale (CSBS)—The CSBS is a semi-structured interaction between an examiner and an infant, designed to assess infants’ communicative and symbolic behavior repertoires. Infants are presented with a series of tempting toys, snacks, and symbolic play opportunities in order to elicit requests and social initiations, including gesture. The CSBS as a standardized measure demonstrates excellent reliability and validity. For the purposes of the present study, the CSBS was used as a context for scoring infant gesture competency.

Autism Diagnostic Observation Schedule: Generic (ADOS-G)—The ADOS is a semi-structured play-based interaction designed to assess participants’ social and communicative abilities across a range of contexts which vary according to language ability. The presence of repetitive behaviors and restricted interests are also noted. Individual items are scored from 0–3, with higher scores indicating more profound impairment. The items in the scoring algorithm map onto DSM- IV criteria for ASD, and empirically-derived cut-offs can be used to categorize scores into those meeting criteria for Autism, Autism Spectrum, or non-spectrum.

Mullen Scales of Early Learning (MSEL)—The MSEL is a standardized developmental assessment designed to be used with infants from birth through 68 months of age. It measures skills in Gross Motor and four cognitive domains: Fine Motor, Visual Reception, Expressive Language and Receptive Language. An 18-month Language score was calculated for each infant by summing their 18-month raw scores on the two language subscales.²

¹Many of the infants included in this study have been seen at 24 or 36 months of age (LRC: 2 at 24 months, 25 at 27 months; HRA: 4 at 18 months, 10 at 24 months, and 33 at 36 months). Whenever available, ADOS scores and clinical judgment from these later visits will be used in making diagnostic classifications. onto DSM- IV criteria for ASD, and empirically-derived cut-offs can be used to categorize scores into those meeting criteria for Autism, Autism Spectrum, or non-spectrum.

²Although many studies of the impact of gesture on language development use a measure of vocabulary such as the MacArthur-Bates Communicative Development Inventory (MCDI; Fenson, Marchman, Thal, Dale, Reznick, & Bates, 2007), this measure was not added until later in the study and therefore was only available for 30 of the 75 infants in this analysis (21 HRA, 9 LRC). Descriptive information on the vocabulary measure of the MCDI (number of words produced) for this subset of infants is presented in table 3 for comparison.

Coding Schemes

Infant Gesture—Infant gesture use was scored from video of the CSBS with an examiner and during the mother-child (MC) interaction. Seven subjects (4 HRA, 3 LRC) did not complete the CSBS due to fussiness. For these participants, gesture use was scored from the mother-child interaction only. All gestures were coded using a scheme developed by Goldin-Meadow and colleagues (Iverson & Goldin-Meadow, 2005; Goldin-Meadow & Mylander, 1984; Rowe and Goldin-Meadow, 2009b) and included communicative use of Deictic (pointing, showing, reaching, etc.), Representational (e.g., using the hands to make a cup or whiskers), and Conventional (e.g. nodding yes, shaking no) gestures. Following Rowe et al., (2008), two final scores were calculated: Tokens and Types. The Tokens score reflects the total number of gestures produced during the session. The Types score reflects the number of different meanings conveyed through gesture. Deictic gestures with different referents were considered to have different meanings. For example, a child pointing twice towards a picture on the wall and once towards a bottle would receive a Token score of 3 and a Type score of 2. To account for differences in session length, scores were calculated as the rate of use per 10 minutes.

Token and Type scores were calculated for each individual context (CSBS and MC) in addition to a total composite score that included all gestures used in either context, yielding 6 total infant gesture variables (Infant CSBS Gesture Tokens and Gesture Types, Infant MC Gesture Tokens and Gesture Types, and Infant Total Gesture Tokens and Gesture Types). These variables were chosen for several important reasons. The first was to more readily enable comparisons between the data collected here and previous reports of the associations between early gesture and language in both typically developing samples which have focused primarily on naturalistic, mother-child interactions, and those reported for young children with autism which have focused more heavily on gestural competency during standardized assessments or on questionnaires. This distinction is also important when considering the relationships between infant and maternal gesture rates which again, have been reported for typically developing dyads in naturalistic mother-child interactions but have not been examined at all in this high risk population. Finally, as gesture production is just beginning to emerge at 12 months of age, the composite variable was created to maximize the observational period and the number of gestures observed; intercorrelations between the two contexts range from $r = .51$ to $r = .70$, suggesting there is some association of gesture production rates across the two contexts.

Maternal Gesture—Maternal gestures were scored from video recording of the 12-month MC interaction using the same procedure outlined for coding of infant gestures, but coded gestures were restricted to those accompanied by specific infant attentional patterns in an effort to ensure they had observed the gestures produced. Deictic gestures were scored only if the infant looked at the referent. Representational and Conventional gestures were scored only if the infant looked towards the mother. Infant attention was coded liberally, and gestures were scored if the infant was either already looking at the target while the gesture was produced, or looked at the target immediately following its production (e.g. were coded whether the gestures were following the infant's focus of attention or trying to direct it, as long as the child looked at the appropriate target) This this approach is consistent with prior

research demonstrating that maternal directives which include gestures and which the infant follows to the target are correlated with infant language (Tomasello & Farrar, 1986). Maternal Gesture Token and Maternal Gesture Type scores (rate per 10 minutes) were calculated.

Maternal Language—Standardized measures of maternal vocabulary size (number of different words, NDW), and overall communicativeness (number of total words, NTW) were derived from transcripts of the MC interaction. The 12-month MC interactions were transcribed by coders trained to 80% reliability for each utterance, using Systematic Analysis of Language Transcripts (SALT) software, in order to obtain a maternal language sample. Due to audio recording errors, transcript information for three mothers was unavailable (2 HRA, 1 LRC). Final scores for each variable reflect the total number of words and number of different words per 10 minutes.

Gesture Coding and Language Transcription—Gestures were coded from videotape using Noldus: The Observer XT software program (version 10.0) by two coders blind to group membership and trained to 80% agreement on each gesture category. Ongoing reliability was maintained by double coding approximately 20% (15) of files and assessed using Intraclass Correlation Coefficients (ICC's). Because both coders scored each of these 15 files, a two-way mixed design was used (Shrout & Fleiss, 1979). ICC's were calculated for each gesture category: Maternal Deictic ($r = .87$), Maternal Conventional ($r = .94$), Maternal Representational ($r = .74$), AC Deictic ($r = .79$), AC Conventional ($r = .83$), AC Representational ($r = .87$), Infant Deictic ($r = .94$), and Infant Conventional ($r = .95$). An ICC for Infant Representational gestures was not computed because no children in either group produced any representational gestures.

Results

Overview

Participants were all seen within a window of 2 weeks prior through 1 month after their 12 and 18-month birthdays, ranging from 11–12 months for the 12 month visit, and from 17–18 months for the 18-month visit. One infant (HRA, female) was excluded from all analyses after it was revealed that the family was not monolingual English-speaking. The mean length of total video footage coded was 25 minutes for infants and 8 minutes for mothers, which did not differ by group (Infant: $t(74) = .512$ $p = .61$; Mother: $t(74) = 2.20$ $p = .90$). Analyses were conducted to address our primary goals. First, to determine whether mothers of infants at risk for ASD differ from mothers of low risk infants in either the overall number of gestures produced, the number of meanings expressed via gesture, or the distribution of gesture types, whether delays in gesture production are present in non-diagnosed infant siblings at 12 months of age and finally, to explore the relationships between these measures. Non-parametric analyses were conducted due to the non-normal distribution (e.g. significant positive skew) exhibited by some of the gesture variables.

Autism Symptoms

Nine infants scored above the cutoff on the ADOS criteria for an ASD at their most recent study visit. Six of these infants both scored above the ADOS threshold for ASD at 36 months and were classified as ASD according to a clinical best estimate rating made by an expert clinician. An additional three infants met criteria for ASD on the ADOS at their most recent study visit (1 at 18, 2 at 24). One of the infants who met ADOS criteria at 24 months has not yet been seen at 36 months. The other two infants who met ADOS criteria at 18 and 24 months months later discontinued their participation in the larger project and a clinical best estimate was made using all available data; both received a clinical best estimate of ASD. For the purposes of the present study, these 9 infants are classified as 'probable' ASD. An additional 4 infants met ADOS criteria at 24 months, but failed to meet ADOS cut-off at 36 months and received clinical judgments of non-ASD. These four infants are included in the larger group of non-ASD high risk infant siblings, designated as HRA.

Group Differences in Maternal Gesture and Language

Descriptive statistics for maternal language and gesture measures are presented in Table 2. To determine whether mothers differed in their rates of gesture and language use at the 12 month laboratory visit, non-parametric analyses (Kruskal-Wallis) were used to evaluate differences between the groups (ASD, HRA, and LRC) for the two gesture variables (Maternal Gesture Tokens, Maternal Gesture Types) and the two language variables (NTW and NDW). The tests for both gesture measures were significant (Maternal Gesture Tokens, $\chi^2(2, N = 74) = 6.70, p = .03$; Maternal Gesture Types $\chi^2(2, N = 74) = 6.20, p < .05$). Post hoc pair-wise comparisons using Mann-Whitney U tests revealed significant differences between HRA and LRC mothers for both Maternal Gesture Tokens ($U = 709.0, p < .01$) and Maternal Gesture Types ($U = 704.0, p = .01$), with HRA mothers producing more of each type. No other contrasts were significant.

Figure 1 displays the distribution of each gesture category for each of the three diagnostic groups. Mothers in each of the three groups produced a majority of deictic gestures (mean_{LRC} = 0.78, SD = 0.19; mean_{HRA} = 0.77, SD = 0.15; mean_{ASD} = 0.78, SD = 0.18), followed by conventional (mean_{LRC} = 0.18, SD = 0.17; mean_{HRA} = 0.17, SD = 0.14; mean_{ASD} = 0.13, SD = 0.15), and representational (mean_{LRC} = 0.04 SD = 0.09; mean_{HRA} = 0.06 SD = 0.08; mean_{ASD} = 0.09, SD = 0.09), respectively. Non-parametric analyses (Kruskal-Wallis) revealed no significant group differences in the proportion of gesture types used by mothers in each of the three groups.

Group Differences in Infant Gesture and Language

Descriptive statistics for infant language and gesture measures are presented in Table 3. Non-parametric analyses (Kruskal-Wallis) were conducted to identify any group differences in infants' gesture use at 12 months of age or language ability at 18 months. The test was significant only for Infant Total Gesture Tokens, $\chi^2(2, N = 74) = 7.287, p = .03$. Post hoc pair-wise comparisons (Mann-Whitney U) revealed significant differences between the ASD infants and both the HRA infants ($U = 94.0, p = .02$.) and LRC infants ($U = 50.5, p = .01$). The comparison for HRA and LRC infants was non-significant ($U = 472.0, p = .59$).

Relationships Between 12-month Gestures and 18-Month Language

Correlational analyses were conducted to examine the association between 12-month measures of infant gesture, maternal gesture and maternal language and 18-month Infant Language scores. Non-parametric (Spearman's) correlations were calculated between each of the 12-month gesture and language predictors (Infant Total Gesture Tokens, Infant Total Gesture Types, Infant MC Gesture Tokens, Infant MC Gesture Types, Infant CSBS Gesture Tokens, Infant CSBS Gesture Types, Maternal Gesture Tokens, Maternal Gesture Types, MLU, and NDW) and infants' 18-month Language scores. Intercorrelations among each of these variables are presented in Table 4.

For all three groups, 18-month Language was significantly correlated with infants' composite gesture scores and CSBS gesture scores. Infant gestures scored from the mother-child interaction were significantly correlated with 18-month language only for the ASD group, and at trend level for the HRA group between Infant MC Gesture Tokens and 18-month Language. 18-month Language was also significantly correlated with maternal gesture use for both the LRC and HRA infants, but not for the ASD group. 18-month Infant Language was not correlated with Maternal NTW or Maternal NDW for any group.

For both HRA and ASD, but not LRC dyads, significant correlations between maternal and infant gestures measures were observed. For HRA dyads, these correlations were present across both the MC and CSBS contexts and in the composite variables. For ASD dyads, these correlations were observed for gestures scored from the CSBS context and for the composite gesture variables.

Partial correlations between Infant 18-month Language and Maternal Gesture Tokens (controlling for Infant Total Gesture Tokens) and Maternal Gesture Types (controlling for Infant Total Gesture Types) were also calculated. Correlations between 18-Month Language and

Maternal Gesture Tokens remained significant for both groups, while the correlation between 18-month Language and Maternal Gesture Tokens remained significant only for the LRC group. (LRC: Maternal Gesture Tokens, $\rho = .43$, $p < .05$, Maternal Gesture Types, $\rho = .51$, $p < .01$; HRA: Maternal Gesture Tokens, $\rho = .34$, $p < .05$).

Discussion

In this study, we examined the frequency and types of gestures used at 12-months of age by infants at risk for autism and their mothers, and the relationship between these early gestures and infants' language scores at 18 months. We found group differences in infant gesture production, with infants later diagnosed with autism producing about half the number of gestures produced by the other two groups. We also found significant group differences for the maternal gesture measures, with mothers of non-diagnosed high risk infants producing more gestures overall and expressing more meanings via gesture than mothers of low risk infants. For low risk and non-diagnosed HRA infants, these maternal gestures were associated with infant language even after controlling for child gesture use.

There were also several surprising findings. Most notably, we did not find a relationship between maternal and infant language in any of the three groups. It is important to note that the majority of parents in both groups have relatively high educational backgrounds and fall within the highest two income brackets, probably related to the time commitment required for participation in the larger longitudinal project. This narrower SES range may be masking some of the effects of maternal language on infant language by focusing only on infants who are receiving relatively high levels of parental verbal input.

Second, maternal gesture production was correlated with infant gesture production for the HRA and ASD, but not LRC dyads, and these associations were observed in both the naturalistic (MC) and semi-structured (CSBS) contexts. There are several possible explanations for these findings. The first is that gesture production is just starting to emerge during this age, and we may not have accurately captured LRC infants' developing gestural abilities as well as those who have focused on slightly older ages for longer observational periods (Iverson, Capirci, Longobardi, & Caselli, 1999; Rowe, Özcalikan, & Goldin-Meadow, 2008). While Liszkowski and colleagues (2012) have recently reported a relationship between rates of maternal and infant pointing between 10 and 14 months, pointing production was scored while parents and infants looked at a complex display but were not permitted to manipulate the objects; thus these findings are not easily compared to the interactive context utilized here. The significance of the broad association between maternal and infant gesture rates is not entirely clear, although it may reflect an increased sensitivity of HRA mothers to the developmental level of their infants, a point we will return to later.

The similar rates of gesture production by the low risk and non-diagnosed groups of infants at 12-months of age are not inconsistent with previous findings, which have not generally documented such delays until 18 months of age or later (Mitchell et al., 2006; Gamliel et al. 2009). The different findings may also reflect differences in the types of gestures we focus on here. In particular, the gesture delays reported by Mitchell et al. (2006) were in the "Late Gestures" domain of the MacArthur-Bates Communicative Development Inventory (CDI) which includes the categories "Actions With Objects", "Pretending to be a Parent", and "Imitating Other Adult Actions" as well as functional play behaviors such as sweeping with a broom or feeding a doll or stuffed animal. These later 'gestures' are qualitatively different from the types of communicative gestures coded here, which include many of the gestures types scored in the "Early Gestures" domain of the CDI, such as pointing and waving hi or bye. These "Early Gestures" were selected specifically for the current study because they have been identified as particularly strong predictors of concurrent and subsequent language ability in both typically developing toddlers and those with ASD (Luyster et al., 2008; Smith et al., 2008; Rowe, Özcalikan, & Goldin-Meadow, 2008).

However, we do replicate the existing literature in documenting delays in gesture production at 12 months of age in infants who later meet criteria for ASD (Mitchell et al., 2006; Osterling & Dawson, 1994). Our results also expand on previous work documenting that even in the presence of early delays in the gestures of infants with ASD, gesture production at 12 months is correlated with language scores 6 months later.

The results of the maternal gesture analyses are less clear, particularly for the ASD group. While it is potentially interesting that the correlations between maternal gesture and 18-month Language are not significant, the magnitude of the correlations themselves is quite similar, and we simply do not have the necessary power to determine whether these are meaningful group differences (Cohen, 1992). Additionally, there are a small number of infants in the ASD group (3) for whom 36-month outcome data is not available, and it is possible that these infants would not be categorized as ASD at 36 months of age, further reducing the number of infants in this group. Nonetheless, the infants in the ASD group considered here represent a group of infants who are demonstrating early difficulties in social communication, and are the very group who would be the targets of early screening and intervention practices. The association between maternal gestures and infants' own gesture production in both high risk groups points to a potential avenue for intervention. However, it is important to note that the analyses presented here do not allow us to adequately determine whether maternal gestures promote infant gesture and language production, or more simply reflects mothers' sensitivity to infants' own abilities. While further work is needed to fully understand the implications of increased rates of gesture production by mothers of non-diagnosed high risk infants, the results obtained here do provide initial evidence that maternal gesture production may be influenced by both global factors such as risk status, and by the social-communicative abilities of individual infants.

The fact that mothers of high risk non-diagnosed infants produce significantly more gestures than mothers of low risk infants - despite the two groups of infants producing gestures with equal frequency - suggests that early parental vigilance and risk status do exert some influence on parental behavior during early dyadic interactions. While an alternative explanation might be that HRA mothers are providing additional communicative support to infants' who are demonstrating subtle communication difficulties such as the response to joint attention difficulties described by Presmanes and colleagues (2007), this interpretation is not supported by the similar rates of gesture production and language ability of the HRA and LRC infants. The analyses presented here do demonstrate that although they gesture more frequently, mothers of HRA infants do not display a relative increase in deictic gestures, as has been reported in other clinical populations (Iverson, Longobardi, Spampinato, & Cristina Caselli, 2006). Thus, this global increase in gesture production by mothers of non-diagnosed high risk infants may be a manifestation of more global effects of risk status, rather than a specific response to infants' impaired communicative abilities. Although group differences in infant gesture were not significant in either of the individual contexts, they were more pronounced in the semi-structured interaction. While this is perhaps unsurprising, given that the task is designed to prompt infants for these types of communicative behaviors, the observed increases in maternal gesture rates by the HRA group may in fact reflect mothers' use of these prompting strategies during naturalistic interactions.

The significance of the gesture production rates observed in mothers of infants with ASD, which do not differ significantly from either the LRC or HRA mothers, is not entirely clear and may simply reflect the limited statistical power afforded by the small number of ASD dyads. If the group differences in maternal gesture production rates do truly differ, this may reflect a specific impact of ASD on early mother-child interactions, with decreased rates of

infant gesture production resulting in decreased rates of reciprocal social interactions, including mother's gestures.

While more work is needed to fully understand these factors, the data presented here take an important first step in understanding how early parent-child interactions may be altered by risk status and symptom emergence, and how these factors influence the early language development of infants at risk for autism.

Acknowledgments

The authors thank the children and families who participated in this study, Susan Goldin-Meadow, PhD. for sharing her coding system with us, our collaborators, Dr. Charles Nelson and Dr. Alice Carter, and the students and staff who assisted in the data collection for this project. This study was supported by grants from the National Institute on Deafness and Other Communication Disorders (NIH R01 DC 010290-01 and R21 DC 08637), Autism Speaks, and the Simons Foundation.

References

- Adamson L, Bakeman R, Deckner D, Ronski M. Joint engagement and the emergence of language in children with autism and Down syndrome. *Journal of Autism and Developmental Disorders*. 2009; 39(1):84–96. [PubMed: 18581223]
- Ben-Yizhak N, Yirmiya N, Seidman I, Alon R, Lord C, Sigman M. Pragmatic language and school related linguistic abilities in siblings of children with autism. *Journal of Autism and Developmental Disorders*. 2011; 41(6):750–760. [PubMed: 20844942]
- Charman T, Baron-Cohen S, Swettenham J, Baird G, Drew A, Cox A. Predicting language outcome in infants with autism and pervasive developmental disorder. *International Journal of Language & Communication Disorders*. 2003; 38(3):265–285. [PubMed: 12851079]
- Cohen J. A power primer. *Psychological bulletin*. 1992; 112(1):155–9. [PubMed: 19565683]
- Colgan SE, Lanter E, McComish C, Watson LR, Crais ER, Baranek GT. Analysis of social interaction gestures in infants with autism. *Child Neuropsychology*. 2006; 12(4–5):307–319. [PubMed: 16911975]
- Dawson G, Meltzoff AN, Osterling J, Rinaldi J, Brown E. Children with autism fail to orient to naturally occurring social stimuli. *Journal of Autism and Developmental Disorders*. 1998; 28(6):479–485. [PubMed: 9932234]
- Fenson, L.; Marchman, VA.; Thal, D.; Dale, PS.; Reznick, JS.; Bates, E. *MacArthur-Bates Communicative Development Inventories: User's guide and technical manual*. 2. Baltimore: Brookes; 2007.
- Gamliel I, Yirmiya N, Jaffe DH, Manor O, Sigman M. Developmental trajectories in siblings of children with autism: Cognition and language from 4 months to 7 years. *Journal of Autism and Developmental Disorders*. 2009; 39(8):1131–1144. [PubMed: 19326200]
- Goldin-Meadow S, Mylander C. Gestural communication in deaf children: The effects and noneffects of parental input on early language development. *Monographs of the Society for Research in Child Development*. 1984; 49(3–4):1–121. [PubMed: 6537463]
- Ibanez LV, Messinger DS, Newell L, Lambert B, Sheskin M. Visual disengagement in the infant siblings of children with an autism spectrum disorder (ASD). *Autism*. 2008; 12(5):473–485. [PubMed: 18805943]
- Iverson JM, Capirci O, Longobardi E, Caselli MC. Gesturing in mother – child interactions. *Cognitive Development*. 1999; 14(1):57–75.
- Iverson JM, Goldin-Meadow S. Gesture paves the way for language development. *Psychological Science*. 2005; 16(5):367–371. [PubMed: 15869695]
- Iverson JM, Longobardi E, Spampinato K, Caselli M. Gesture and speech in maternal input to children with Down's syndrome. *International Journal Of Language & Communication Disorders*. 2006; 41(3):235–251.10.1080/13682820500312151 [PubMed: 16702092]

- Iverson JM, Wozniak RH. Variation in vocal-motor development in infant siblings of children with autism. *Journal of Autism and Developmental Disorders*. 2007; 37(1):158–170. [PubMed: 17191097]
- Landry R, Bryson SE. Impaired disengagement of attention in young children with autism. *Journal of Child Psychology and Psychiatry*. 2004; 45(6):1115–1122. [PubMed: 15257668]
- Liszkowski U, Brown P, Callaghan T, Takada A, De Vos C. A prelinguistic gestural universal of human communication. *Cognitive Science*. 2012 Advance online publication. 10.1111/j.1551-6709.2011.01228.x
- Liszkowski U, Tomasello M. Individual differences in social, cognitive, and morphological aspects of infant pointing. *Cognitive Development*. 2011; 26:16–29.10.1016/j.cogdev.2010.10.001
- Lord C, Risi S, Lambrecht L, Cook E Jr, Leventhal B, DiLavore P, et al. The Autism Diagnostic Observation Schedule — Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*. 2000; 30(3):205–223. [PubMed: 11055457]
- Luyster RJ, Kadlec MB, Carter A, Tager-Flusberg H. Language assessment and development in toddlers with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. 2008; 38(8):1426–1438. [PubMed: 18188685]
- McMahon C, Malesa E, Yoder P, Stone W. Parents of children with autism spectrum disorders have merited concerns about their later-born infants. *Research & Practice for Persons with Severe Disabilities*. 2007; 32(2):154–160.
- Mitchell S, Brian J, Zwaigenbaum L, Roberts W, Szatmari P, Smith I, et al. Early language and communication development of infants later diagnosed with autism spectrum disorder. *Journal of Developmental and Behavioral Pediatrics*. 2006; 27:S69–S78. [PubMed: 16685188]
- Mullen, EM. AGS. Mullen Scales of Early Learning. Circle Pines, MN: American Guidance Service Inc; 1995.
- Nadig AS, Ozonoff S, Young GS, Rozga A, Sigman M, Rogers SJ. A prospective study of response to name in infants at risk for autism. *Archives of Pediatrics Adolescent Medicine*. 2007; 161(4):378–383. [PubMed: 17404135]
- Osterling J, Dawson G. Early recognition of children with autism: A study of first birthday home videotapes. *Journal of Autism and Developmental Disorders*. 1994; 24(3):247–257. [PubMed: 8050980]
- Ozonoff S, Iosif A, Baguio F, Cook IC, Hill MM, Hutman T, et al. A prospective study of the emergence of early behavioral signs of autism. *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010; 49(3):256–266. [PubMed: 20410715]
- Ozonoff S, Young GS, Steinfeld M, Hill MM, Cook I, Hutman T, Sigman M. How early do parent concerns predict later autism diagnosis? *Journal Of Developmental And Behavioral Pediatrics*. 2009; 30(5):367–375. [PubMed: 19827218]
- Paul R, Fuerst Y, Ramsay G, Chawarska K, Klin A. Out of the mouths of babes: Vocal production in infant siblings of children with ASD. *Journal of Child Psychology and Psychiatry*. 2011; 52(5):588–598. [PubMed: 21039489]
- Presmanes AG, Walden TA, Stone WL, Yoder PJ. Effects of different attentional cues on responding to joint attention in younger siblings of children with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. 2007; 37(1):133–144. [PubMed: 17186366]
- Rowe ML, Goldin-Meadow S. Differences in early gesture explain SES disparities in child vocabulary size at school entry. *Science*. 2009; 323(5916):951–953.10.1126/science.1167025 [PubMed: 19213922]
- Rowe ML, Goldin-Meadow S. Early gesture selectively predicts later language learning. *Developmental Science*. 2009; 12(1):182–187. [PubMed: 19120426]
- Rowe ML, Özçaliskan S, Goldin-Meadow S. Learning words by hand: Gesture's role in predicting vocabulary development. *First Language*. 2008; 28(2):182–199. [PubMed: 19763249]
- Rozga A, Hutman T, Young GS, Rogers SJ, Ozonoff S, Dapretto M, et al. Behavioral profiles of affected and unaffected siblings of children with autism: Contribution of measures of mother – infant interaction and nonverbal communication. *Journal of Autism and Developmental Disorders*. 2011; 41(3):287–301. [PubMed: 20568002]

- Shrout PE, Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin*. 1979; 86(2):420–428. [PubMed: 18839484]
- Shumway S, Wetherby AM. Communicative acts of children with autism spectrum disorders in the second year of life. *Journal of Speech, Language, and Hearing Research*. 2009; 52(5):1139–1156.
- Sigman M, McGovern CW. Improvement in cognitive and language skills from preschool to adolescence in autism. *Journal of Autism and Developmental Disorders*. 2005; 35(1):15–23. [PubMed: 15796118]
- Smith V, Mirenda P, Zaidman-Zait A. Predictors of expressive vocabulary growth in children with autism. *Journal of Speech, Language, And Hearing Research*. 2007; 50(1):149–160.10.1044/1092-4388(2007/013)
- Stone WL, McMahon CR, Yoder PJ, Walden TA. Early social-communicative and cognitive development of younger siblings of children with autism spectrum disorders. *Archives of Pediatrics and Adolescent Medicine*. 2007; 161:384–390. [PubMed: 17404136]
- Tager-Flusberg, H.; Paul, R.; Lord, C. Language and communication in autism. In: Volkmar, FR., et al., editors. *Handbook of autism and pervasive developmental disorders*, vol. 1: Diagnosis, development, neurobiology, and behavior. 3. Hoboken, NJ US: John Wiley & Sons Inc; 2005. p. 335-364.
- Thurm A, Lord C, Lee L, Newschaffer C. Predictors of language acquisition in preschool children with autism spectrum disorders. *Journal of Autism and Developmental Disorders*. 2007; 37(9):1721–1734. [PubMed: 17180717]
- Tomasello M, Farrar MJ. Joint attention and early language. *Child Development*. 1986; 57(6):1454–1463. [PubMed: 3802971]
- Toth K, Munson J, Meltzoff AN, Dawson G. Early predictors of communication development in young children with autism spectrum disorder: Joint attention, imitation, and toy play. *Journal of Autism and Developmental Disorders*. 2006; 36(8):993–1005. [PubMed: 16845578]
- Wan M, Green J, Elsabbagh M, Johnson M, Charman T, Plummer F. The BASIS Team. Parent-infant interaction in infant siblings at risk of autism. *Research In Developmental Disabilities*. 2012; 33(3):924–932.10.1016/j.ridd.2011.12.011 [PubMed: 22257674]
- Wetherby, A.; Prizant, B. *Communication and Symbolic Behavior Scales-Normed Edition*. Baltimore: Paul H. Brookes; 1993.
- Werner E, Dawson G, Osterling J, Dinno N. Brief report: Recognition of autism spectrum disorder before one year of age: A retrospective study based on home videotapes. *Journal of Autism and Developmental Disorders*. 2000; 30(2):157–162. [PubMed: 10832780]
- Yirmiya N, Gamliel I, Pilowsky T, Feldman R, Baron-Cohen S, Sigman M. The development of siblings of children with autism at 4 and 14 months: Social engagement, communication, and cognition. *Journal of Child Psychology and Psychiatry*. 2006; 47(5):511–523. [PubMed: 16671934]
- Yirmiya N, Gamliel I, Shaked M, Sigman M. Cognitive and verbal abilities of 24 to 36-month-old siblings of children with autism. *Journal of Autism and Developmental Disorders*. 2007; 37:218–229. [PubMed: 16897384]
- Yoder P, Stone WL, Walden T, Malesa E. Predicting social impairment and ASD diagnosis in younger siblings of children with autism spectrum disorder. *Journal of Autism and Developmental Disorders*. 2009; 39(10):1381–1391. [PubMed: 19449096]
- Yoder PJ, Warren SF. Relative treatment effects of two prelinguistic communication interventions on language development in toddlers with developmental delays vary by maternal characteristics. *Journal Of Speech, Language, And Hearing Research*. 2001; 44(1):224–237.
- Zwaigenbaum L, Bryson S, Rogers T, Roberts W, Brian J, Szatmari P. Behavioral manifestations of autism in the first year of life. *International Journal of Developmental Neuroscience*. 2005; 23(2–3):143–152. [PubMed: 15749241]
- Zwaigenbaum L, Thurm A, Stone W, Baranek G, Bryson S, Iverson J, et al. Studying the emergence of autism spectrum disorders in high-risk infants: Methodological and practical issues. *Journal of Autism and Developmental Disorders*. 2007; 37(3):466–480. [PubMed: 16897376]

Figure 1.
Proportion of each maternal gesture type

Table 1Participant Demographics, by Enrollment Group^a

	Group	
	Low Risk (<i>n</i> =27)	High Risk (<i>n</i> =47)
Gender (% male)	52.0	55.3
Ethnicity/Race (% non-White, non-Hispanic)	24.1	8.5
Family Income level (% with income less than \$65,000)	14.9	18.5
12 Month Nonverbal Developmental Quotient ^b (mean, SD)	128.3 (15.2)	122.4 (13.1)
Chronological age (days) at 12 month visit (mean, SD)	368.7 (10)	372.1 (9)
Chronological age (days) at 18 month visit (mean, SD)	556.3 (9)	557.2 (13)

^aNote that the data presented here includes the subset of children with ASD outcomes

^bNonverbal Developmental Quotients reflect infants' performance on the Visual Reception and Fine Motor subscales of the Mullen Scales of Early Learning (MSEL), adjusted for chronological age.

Table 2

Means and Standard Deviations for Maternal Gesture and Language Measures, by Group

	Group		
	<u>LRC</u> (n= 27)	<u>HRA</u> (n= 38)	<u>ASD</u> (n= 9)
Maternal Gesture Tokens Mean (SD)	14.78 (7.2)	20.85 (8.8) **	17.58 (13.9)
Maternal Gesture Types Mean (SD)	10.93 (5.4)	14.27 (5.4) *	12.77 (8.4)
Maternal 12-month NTW Mean (SD)	486.64 (148.9)	479.88 (213.2)	531.53 (237.0)
Maternal 12-month NDW Mean (SD)	151.22 (35.0)	148.60 (55.2)	139.18 (57.36)

**
 $p < .01$,

*
 $p < .05$ for HRA versus LRC contrast

Table 3

Means, Standard Deviations, and Range for Infant Gesture and Language Measures, by Group

	Group		
	<u>LRC</u> (n= 27)	<u>HRA</u> (n= 38)	<u>ASD</u> (n= 9)
Composite Infant Gesture Measures			
Infant Total Gesture Tokens			
Mean (SD)	6.26 (5.0)	5.65 (5.9)	2.49 (3.9) *
Range	18.30	34.50	11.70
Infant Total Gesture Types			
Mean (SD)	3.93 (2.9)	3.71 (3.0)	1.97 (2.9)
Range	11.44	16.60	8.88
Context-Specific Infant Gesture Measures			
Infant MC Gesture Tokens			
Mean (SD)	2.78 (2.7)	2.90 (4.9)	2.19 (3.7)
Range	10.74	23.10	11.13
Infant MC Gesture Types			
Mean (SD)	1.90 (1.9)	2.12 (2.9)	1.66 (2.6)
Range	6.14	14.70	7.41
Infant CSBS ^b Gesture Tokens			
Mean (SD)	6.32 (5.8)	5.22 (6.9)	1.95 (2.9)
Range	21.89	38.68	8.57
Infant CSBS Gesture Types			
Mean (SD)	3.95 (3.4)	3.19 (3.3)	1.66 (2.4)
Range	12.89	17.00	7.14
Infant Language Measures			
Infant 18-month Language Score			
Mean (SD)	37.74 (4.36)	36.53 (6.4)	34.33 (8.9)
Range	16	31	27
Infant 18-month MCDI Words Produced			
Mean (SD)	74.56 (49.7)	89.62 (103.5)	153.60 (204.5)
Range	138	359	466

* p< .05 for ASD vs. LRC and ASD vs. HRA contrasts

^a Note that infant gesture scores from the CSBS do not include the small number of infants who did not complete this assessment (3 LRC, 4 HRA).

^b MCDI scores are reported from a subset of infants (9 LRC, 16 HRA, 5 ASD)

Table 4
Zero-order Spearman's correlations of Infant and Maternal 12-Month Gesture Scores, by Group

	1	2	3	4	5	6	7	8	9	10	11
LRC											
1 Infant 18-month Language Score	-										
2 Infant Total Gesture Tokens	.60**	-									
3 Infant Total Gesture Types	.59**	.97**	-								
4 Infant MC Gesture Tokens	.17	.60**	.51**	-							
5 Infant MC Gesture Types	.23	.71**	.66**	.96**	-						
6 Infant CSBS Gesture Tokens	.64**	.91**	.84**	.52**	.61**	-					
7 Infant CSBS Gesture Types	.62**	.92**	.91**	.51*	.62**	.96**	-				
8 Maternal Gesture Tokens	.50**	.15	.14	.08	.13	.19	.17	-			
9 Maternal Gesture Types	.58**	.18	.19	.05	.10	.22	.25	.92**	-		
10 Maternal 12-month NDW	.31	.53**	.55	.31	.38	.46*	.49*	-.02	.14	-	
11 Maternal 12-month NTW	.01	.32	.25	.41*	.38	.25	.19	.03	.04	.73	-
	1	2	3	4	5	6	7	8	9	10	11
HRA											
1 Infant 18-month Language Score	-										
2 Infant Total Gesture Tokens	.37*	-									
3 Infant Total Gesture Types	.34*	.97**	-								
4 Infant MC Gesture Tokens	.28†	.71**	.70**	-							
5 Infant MC Gesture Types	.23	.70**	.72**	.98**	-						
6 Infant CSBS Gesture Tokens	.43*	.88**	.86**	.69**	.70**	-					
7 Infant CSBS Gesture Types	.33†	.87**	.88**	.71**	.72**	.97**	-				
8 Maternal Gesture Tokens	.38*	.34*	.28†	.31†	.36*	.25	.25	-			
9 Maternal Gesture Types	.30†	.34*	.35*	.37*	.41*	.32†	.33†	.78**	-		
10 Maternal 12-month NDW	.13	-.05	-.01	.09	.12	.04	.05	.19	.30†	-	
11 Maternal 12-month NTW	.08	-.02	.03	.07	.11	-.00	.02	.17	.26	.89**	-
	1	2	3	4	5	6	7	8	9	10	11

	1	2	3	4	5	6	7	8	9	10	11
ASD	1	Infant 18-month Language Score	-	-	-	-	-	-	-	-	-
2	Infant Total Gesture Tokens	.84**	-	-	-	-	-	-	-	-	-
3	Infant Total Gesture Types	.83**	.99**	-	-	-	-	-	-	-	-
4	Infant MC Gesture Tokens	.81**	.97**	.97**	-	-	-	-	-	-	-
5	Infant MC Gesture Types	.76**	.94**	.95**	.99**	-	-	-	-	-	-
6	Infant CSBS Gesture Tokens	.80**	.90**	.89**	.76*	.70*	-	-	-	-	-
7	Infant CSBS Gesture Types	.83**	.95**	.95**	.85**	.80**	.98**	-	-	-	-
8	Maternal Gesture Tokens	.29	.60 [†]	.61 [†]	.53	.54	.60 [†]	.58	-	-	-
9	Maternal Gesture Types	.26	.57	.58	.49	.51	.57	.55	.99**	-	-
10	Maternal 12-month NDW	.25	.42	.42	.38	.40	.42	.37	.64	.67	-
11	Maternal 12-month NTW	.44	.54	.54	.50	.48	.60	.53	.82*	.82*	.90*

[†] p<.10,

* p<.05,

** p<.01, all two-tailed