A PROSPECTIVE LONGITUDINAL INVESTIGATION OF EARLY PRECURSORS OF SOCIAL AND NON-SOCIAL AUTISTIC TRAITS IN A COMMUNITY SAMPLE OF 18-MONTH-OLD SINGAPOREAN TODDLERS.

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DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety.

I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

GAN ZHENG QIANG, DANIEL

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THESIS SUMMARY

Background: Recent research has demonstrated that while Autism Spectrum Disorders (ASDs) and autistic-like traits (ALTs) share a common etiology, differences may exist in the underlying etiology of the different core autistic dimensions. Studying known infant precursors of ASD in relation to different clusters of dimensionally measured ALTs in unselected samples is a conceptually relevant and methodologically sound approach for elucidating current understanding of the causes of ASD/ALTs. Furthermore, few studies have examined the predictive utility of early precursors on later ALTs in non-Western contexts.

Aims: This thesis investigated whether early precursors of ASD from gestation up to the first year of life (pregnancy/birth complications, infant temperament at 3 months, and 12-month social development –imitation/play, gestures, and empathy) were significantly associated with and predicted later social and non-social ALTs in 18-month-old toddlers. In doing so, it concurrently explored the extent to which previous research on early precursors of ASD/ALTs, which have all been conducted in Western-based populations, can be generalized to an unselected Asian community sample.

Method: Participants were 368 Singaporean toddlers involved in a larger prospective longitudinal study: GUSTO (Growing Up in Singapore Towards healthy Outcomes). Information on pregnancy/birth complications were obtained from standardized inventories. Caregivers completed measures of temperament, social development and autistic traits at 3, 12, and 18 months respectively. Hierarchical regression analyses controlling for demographic covariates were conducted to identify significant predictors of later social and non-social ALTs.

Results: The key findings of this study were that early infancy precursors within the first 12 months of life were predictive of ALTs at 18 months and that social and non-social ALTs

were associated with and predicted by different precursors. Importantly, different infant precursors in the first year of life predicted social and non-social ALTs at 18 months. Pregnancy/birth complications, imitation, and empathy were not associated with later ALTs.

Discussion: The study findings resonate with earlier literature suggesting that the core autistic dimensions are each underpinned by distinct sets of etiological factors. In particular, the fact that social and non-social ALTs are associated with different infant precursors suggests that they are probably associated with different early neurodevelopmental processes. This thesis provided preliminary cross-cultural evidence supporting the view that the etiological contributions and neurobiological abnormalities underpinning the different core autistic dimensions are likely different.

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LIST OF ACRONYMS AND ABBREVIATIONS

ASD	Autism Spectrum Disorder
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ALTs	Autistic-like Traits
BAP	Broader Autism Phenotype
CTS	Carey Temperament Scales
EITQ	Early Infancy Temperament Questionnaire
GUSTO	Growing Up in Singapore Towards healthy Outcomes
ITSEA	Infant—Toddler Social and Emotional Assessment
LBW	Low Birth Weight
PPOs	Prenatal, perinatal, and obstetric complications
RRBIs	Restricted, repetitive behaviours and interests
SECDI	Singapore Early Communicative Development Inventories

CHAPTER 1

INTRODUCTION

1.1. Overview

Autism Spectrum Disorder (ASD) is a group of complex, heterogeneous neurodevelopmental conditions characterized by impairments in reciprocal social interaction and communication (i.e. poor use of gesturing or pointing, reduced orienting to name, and inability to interpret and/or respond appropriately to social cues) and patterns of circumscribed behaviours and interests (i.e. self-stimulatory behaviours such as hand-flapping, preoccupations with unconventional objects/interests, and insistence on sameness), with symptoms present in the early developmental period (American Psychiatric Association, 2013). ASDs affect approximately 6-7 per 1000 children worldwide (Kuehn, 2007; Newschaffer et al., 2007; see also Fombonne, 2005, for a review), and about 15 per 10000 children in Asia (Sun & Allison, 2010). ASDs are four times as prevalent in males as in females, and occur across the full spectrum of intellectual ability (Fombonne, 2006). Individuals with ASD vary widely in terms of the severity, presentation and impact of their symptoms.

ASDs have been found to be comorbid with a range of medical and genetic conditions, such as epilepsy (Fombonne, 2003), tuberous sclerosis (Spence, 2004) and Fragile X syndrome (Fombonne, 2005). They have also been frequently associated with psychiatric and behavioural difficulties, such as higher rates of internalizing and externalizing difficulties and emotional regulation problems (Rieffe et al., 2011), as well as sensory and perceptual

impairments (Reynolds & Lane, 2008). A diagnosis of ASD involves thorough gathering of information on the child's developmental history, and detailed observations of the child in multiple contexts. These observations are guided by the use of well-validated clinical interviews, such as the Autism Diagnostic Interview-Revised (ADI-R; Lord, Rutter, & Couteur, 1994), and semi-structured clinical observation methods, such as the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter, DiLavore, & Risi, 1999).

Causal explanations for ASD remain elusive despite considerable advancements in the field. High heritability estimates for ASD have consistently been reported in numerous twin and family studies—ranging from 60% to 90% (Bailey et al., 1995)—indicating that genetic factors play a major role in the etiology of ASD. However, the lack of complete concordance between monozygotic twins suggests that early environmental influences likely interact with existing genetic vulnerabilities in altering the typical trajectory of neurodevelopment in a way that leads to the emergence of autistic symptoms (Abrahams & Geschwind, 2008; Lauritsen & Ewald, 2001).

Autistic-Like Traits (ALTs) are subclinical presentations of the social interaction, communication, and circumscribed behaviours and interests which are not sufficiently severe to warrant a formal clinical diagnosis of ASD (Constantino & Todd, 2003). First evidence that non-autistic individuals do display ALTs was documented by Folstein and Rutter (1977), who coined the term "Broader Autism Phenotype" (BAP) to describe the range of autistic-like behaviours/traits that were observed in relatives of individuals with ASD (Hurley, Losh, Parlier, Reznick, & Piven, 2007; Piven, Palmer, Jacobi, Childress, & Arndt, 1997). Elevated rates of ALTs have been observed in

first-degree relatives of individuals diagnosed with ASD, compared to first-degree relatives of individuals without ASD (Bishop, Maybery, Wong, Maley, & Hallmayer, 2006; Dawson et al., 2002), suggesting that the heritable influences underlying ASD likely contribute to sub-threshold ALTs as well. Importantly, more recent studies which measured ALTs in community samples have found ALTs to be continuously distributed in the general population (Constantino & Todd, 2003; Plomin, Haworth, & Davis, 2009). In light of accumulating evidence that ALTs and ASD are underpinned by common etiological factors (see Ronald & Hoekstra, 2011, for a review), it has been proposed that elucidating the causes of normally-distributed ALTs in the community may pave the way for a better understanding of the etiology of ASD (Ronald & Hoekstra, 2014).

Studying the relationships between early precursors and dimensionally measured ALTs in unselected community samples may provide a potentially fruitful avenue for gaining insight into the impact of causative influences underlying the later manifestation of ALTs (Gerdts & Bernier, 2011). Findings obtained from typical populations may then provide the basis for testing out and investigating specific hypotheses for confirmation and extrapolation in clinical populations (see Section 1.2.1 for further discussion). In addition, employing a dimensional approach is better aligned with present understanding of autistic traits as a continuous construct, with individuals diagnosed with ASD being at the extreme end of the same continuum (Lundström et al., 2012). Moreover, this approach affords several methodological advantages over earlier studies which have adopted the traditional categorical approach (case-control comparisons) to understanding

the associations between early precursors and later risk of ASD (see section 1.2.2 for a detailed discussion).

Recent research indicates that each cluster of ALTs may be caused by distinct sets of etiological factors. Multiple population-based twin studies have consistently found that the genetic and environmental etiological influences underlying each of the core autistic dimensions were only modestly correlated, and that this was stable throughout the autism severity spectrum (see Ronald and Hoekstra, 2014, for a review). In addition, factor analytic studies generally converge on the view that ASD/ALTs are comprised of multiple distinct factors rather than a single underlying construct (see Shuster, Perry, Bebko, & Toplak, 2014, for a review); this provides further evidence suggesting that the etiological causes underlying each construct are possibly different. Considered together, these findings suggest that the constituent clusters of autistic traits/symptoms are phenotypically and etiologically distinct. Importantly, these findings have been recognized in the DSM-5, which currently adopts a two-factor symptom model consisting of a social-communication factor, and a restricted repetitive behaviours and interests (RRBIs) factor (American Psychiatric Association, 2013). Empirical evidence exists in support of the validity of this dyadic model, as well as its superiority over the triadic symptom model employed in the DSM-4 (Mandy, Charman, & Skuse, 2012). Hence, it is recommended that associations between the hypothesized early precursors and later ALTs be studied in relation to specific clusters of ALTs (Ronald & Hoekstra, 2014).

This thesis sought to prospectively investigate whether variables thought to be possible early precursors of ASD were significantly associated

with and predicted later social and non-social/behavioural ALTs in an unselected community sample of Singaporean toddlers. Understanding the contributions of possible precursors to specific clusters of ALTs could pave the way for a better understanding of the impact of etiological factors on the trajectory of neurodevelopment for specific autistic symptoms. While retrospective studies have found that behavioural or developmental peculiarities suggestive of later ASD/ALTs emerge as early as within the first of life (Baranek, 1999; Bolton, Golding, Emond, & Steer, 2012; Osterling & Dawson, 1994), few studies have prospectively examined early markers before 12 months of age, and no study has examined early precursors separately in relation to the constituent clusters of autistic traits. Furthermore, no study has investigated the predictive utility of early infant precursors on later ASD/ALTs in an Asian context. Thus, there is also a need to investigate the extent to which the associations between early markers and ASD/ALTs found in Western-based studies can be extended to Asian populations. The present work aimed to address these gaps.

The literature review that follows critically summarizes (i) the rationale for and methodological advantages of using a dimensional rather than categorical approach to examining infant precursors of ASD (section 1.2), (ii) the need to study predictors of the different autistic core dimensions separately (section 1.3), and (iii) a review of early precursors (within the first year of life) that have been found to be associated with higher risk of ASD and/or higher levels of later ALTs: namely, pre-, perinatal, and obstetric complications (section 1.4), infant temperament (section 1.5), and infant social

development (section 1.6). These sections are then followed by (iv) the present study's aims, research questions and hypotheses (section 1.7).

1.2. Studying early precursors of ALTs dimensionally

1.2.1. Evidence supporting the use of a dimensional approach

Autistic symptoms were initially conceptualized as a discrete repertoire of qualitatively unique behavioural impairments (Rutter & Schopler, 1987) and hypothesized to be caused by a specific set of etiological influences (Rutter, 1978). However, emerging evidence has pointed towards the contrary on both counts. ALTs have been found to be continuously distributed in the general population, with individuals with ASD lying at the quantitative extreme end of this continuum (Kanne, Christ, & Reiersen, 2009; Ronald, Happé, & Plomin, 2005). Furthermore, sub-threshold ALTs and clinically significant autistic symptoms have been found to be underpinned by shared etiological factors (Lundström et al., 2012; Robinson et al., 2011). These findings have led to the reconceptualization of autism as a dimensional construct, as opposed to a condition with a discrete set of symptoms that present exclusively in individuals who meet the diagnostic criteria for ASD.

Research exploring the distribution of ALTs in population-based samples and in factor-analytic studies comparing the factor structures of ASD and ALTs both provide converging evidence in support of the dimensionality of the autism construct. Quantitative assessment of autistic traits in community samples of children (Constantino & Todd, 2003; Williams et al., 2008) and adults (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Hoekstra, Bartels, Verweij, & Boomsma, 2007) have consistently found ALTs

to be normally distributed in the general population, with individuals with ASD lying at the quantitative extreme end of the distribution. In addition, studies which examined the factor structure of ALTs in unselected samples (Allison et al., 2010; Williams et al., 2008) and in clinical samples of children diagnosed with ASD (Frazier, Youngstrom, Kubu, Sinclair, & Rezai, 2008; Gotham, Risi, Pickles, & Lord, 2007) report that there is no evidence suggesting that clinical ASD symptoms and sub-threshold ALTs are distinct constructs; the two differ only in terms of severity.

A wealth of research has highlighted the presence of an etiological link between ALTs in the general population and individuals with a diagnosis of ASD. Early evidence in support of a shared etiology stemmed from observations that non-autistic relatives of individuals diagnosed from ASD often exhibit subclinical autistic-like behaviours and traits (Bolton et al., 1994; Constantino et al., 2006; Folstein & Rutter 1977; Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985; Piven, Palmer, Jacobi, Childress, & Arndt, 1997; Virkud et al., 2009), suggesting that the heritable influences implicated in ASD are also associated with sub-threshold ALTs.

Recent population-based twin studies have yielded congruent findings. Two large studies in the United Kingdom (UK), based on the Twins' Early Development Study (TEDS), assessed the degree of etiological overlap between ALTs at the normal and extreme ranges at two time-points—when twins were 8 years of age (Ronald, Happé, Price, Baron-Cohen, & Plomin, 2006) and again when they reached 12 years of age (Robinson et al., 2011)—using dimensional measures of autistic traits. Both studies reported moderate to high heritability and modest shared environmental contributions in relation

to autistic trait variability. Importantly, no differences in etiological contributions to ALT variability were found between (i) the quantitative extreme and the general population (Ronald et al., 2006b), and (ii) among different subgroups (top 1%, 2%, and 5%) within the extreme range (Robinson et al., 2011). These findings were replicated in a Swedish nationwide twin cohort (N = 19208) of 9- and 12-year-old children from the Child and Adolescent Twin Study (CATSS; Lundström et al., 2012). Furthermore, two early childhood studies have reported moderate genetic and modest environmental contributions to dimensionally measured autistic traits in 2- to 3-year-old twin pairs (Edelson & Saudino, 2009; Stilp, Gernsbacher, Schweigert, Arneson, & Goldsmith, 2010), highlighting that etiological contributions to autistic trait variation are relatively stable from as early as late infancy/early toddlerhood.

Finally, evidence of a common etiology has also been demonstrated in terms of the similar associations that ALTs and ASD share with other comorbid conditions or symptoms, such as ADHD (Leyfer et al., 2006; Reiersen, Constantino, Grimmer, Martin, & Todd, 2008), as well as affective and anxiety-related disorders (Bolton, Pickles, Murphy, & Rutter, 1998).

In summary, several strands of evidence show support for the dimensionality of the autism construct by demonstrating the etiological and phenotypical link between ASD and ALTs. This implies that findings obtained from studying the variability of ALTs in the general population can then serve as a basis for the formation of more specific hypotheses which, in turn, can be tested in clinical populations and employed to further our understanding of the

potential causes or mechanisms/processes leading to clinically significant ASD symptoms.

1.2.2. Methodological strengths of studying ALTs dimensionally in the general population

Past research investigating the associations between early markers of ASD and later risk of ASD largely consist of two types of studies: (i) retrospective comparisons of the early development of children who later develop ASD versus those who do not (Adrien et al., 1993; Baranek, 1999; Osterling and Dawson, 1994; see also Palomo, Belinchon & Ozonoff, 2006, for a review), and (ii) prospective studies which track and compare the early development of "high-risk infant siblings"—baby siblings of older children with ASD, who are at higher risk of developing ASD—with siblings of older children without ASD (Rogers, 2009; Zwaigenbaum et al., 2009). These studies have contributed greatly to the identification of early developmental precursors associated with later ASD. However, they usually have rather limited statistical power for detecting differences owing to small sample sizes. In addition, prospective studies generally involve the expenditure of a disproportionately large amount of financial and manpower-related sources, in comparison to the eventual size of the sample eligible for study. For example, in studies tracking the early development of high-risk infant siblings, approximately 100 siblings need to be extensively followed up individually over time in order to obtain a sample of approximately 20 who will eventually be diagnosed with ASD (Ozonoff et al., 2011).

Employing a dimensional approach to studying early precursors of ALTs affords a number of methodological advantages. Firstly, this approach allows relationships between traits associated with clinical conditions and their risk factors to be studied in larger community samples. In light of evidence that ALTs and ASD have a shared etiology, ALT severity is expected to vary continuously among community participants. This is important as the recruitment of larger samples from the general population is more feasible than from clinical populations. This affords greater opportunity for achieving sufficient statistical power, which is essential for the detection of more subtle relationships or effects.

Secondly, studying early precursors in large community samples allows multiple precursors to be studied in relation to each other, allowing the development of more complex multi-etiological models. In comparison, previous prospective studies utilizing clinical samples have been restricted in terms of the number of factors they could study, owing to small sample sizes (Lord, Mulloy, Wendelboe & Schopler, 1991; Piven et al., 1993).

Finally, the dimensional approach is aligned with evidence in support of a continuous (dimensional) rather than a dichotomous (categorical) relationship between risk factors/precursors and psychopathological symptoms (Hudziak, Achenbach, Althoff, & Pine, 2007). In the context of ALTs, variability in the types and degrees of ALTs among children in the general population might possibly be explained by variance in the assortment and strength of the influence of early markers of ALTs during infancy. This may be helpful and informative in examining potentially more complex relationships that account for the additive effects of multiple precursors.

1.3. The need to study early precursors in relation to the different clusters of ALTs

Research examining the predictive utility of early precursors on later ASD/ALTs has so far been predicated on the assumption that the same etiological influences underlie all of the core autistic traits/symptoms (Happé, Ronald, & Plomin, 2006). However, increasing evidence supports the view that distinct sets of etiological factors, each having their own unique impact on early neurodevelopmental processes, underpin each cluster of ALTs.

Three population-based twin studies by Ronald and colleagues, all utilizing participants from the TEDS cohort, examined the degree of etiological and phenotypic overlap among the triad of core autistic symptoms outlined in the DSM-IV: reciprocal social deficits, communication deficits, and RRBIs (American Psychiatric Association, 2000). Ronald, Happé and Plomin (2005) employed multivariate genetic model fitting analyses to evaluate the extent of genetic overlap between social and non-social autistic behaviours in a large sample of approximately 3400 7-year-old twin pairs. They reported modest genetic correlations between social and non-social ALTs (r_g = .07—.40) and the absence of a strong correlation between social and non-social domain scores within individuals, suggesting little etiological and phenotypic overlap between social and non-social ALTs (Ronald et al., 2005). A year later, two similar investigations were simultaneously conducted on the same sample of twin pairs (Ronald et al., 2006a), and on a subsample who obtained extreme scores (the highest scoring 5%) (Ronald et al., 2006b). A dimensional measure of autistic traits was used to quantify ALTs in both

these studies. While both studies reported high heritability estimates at the normal and extreme ranges within each trait cluster, genetic and phenotypic overlap between different trait clusters were modest regardless of the level of severity (Ronald et al., 2006a, 2006b). In light of these findings, Happé and Ronald (2008) proposed the 'Fractionable Autism Triad' hypothesis, a theory postulating that different clusters of ALTs/autistic symptoms are phenotypically and etiologically 'fractionable': that is, that each cluster is symptomatically distinct and governed by a different set of genetic and environmental etiological factors.

Two recent twin studies have tested the predictions of the 'Fractionable Autism Triad' hypothesis. Ronald, Larsson, Anckersater & Lichtenstein (2011) evaluated the degree of genetic and environmental overlap between different ALTs in a Swedish epidemiological cohort of over 6000 twin pairs aged 9 or 12 years old. In addition, Robinson and colleagues (2012) assessed whether the findings of the earlier described series of studies by Ronald and colleagues (2005, 2006a, 2006b) could still be observed in participants from the TEDS at 12 years of age. Consistent with past findings, both studies reported little overlap of genetic and environmental influences among the three dimensions of the autistic triad (Robinson et al., 2012; Ronald et al., 2011), thereby supporting the predictions of the Fractionable Autism Triad hypothesis.

Factor analytic studies of measures of autistic symptoms in children provide further evidence that the core autistic dimensions are underpinned by different sets of etiological factors. The vast majority of these studies did not

find autistic symptoms to load onto a single factor, yielding multiple-factor solutions instead (for reviews, see: Happé & Ronald, 2008; Mandy & Skuse, 2008). Although there is significant variability in the factor structures obtained across studies—the studies reviewed in Shuster et al. (2014) proposed two- to five-factor solutions—the general consensus of a multiple-factor solution nevertheless has significant implications pertaining to the etiology of autistic symptoms/traits. If autism were indeed a unitary construct and autistic symptoms were all underpinned by the same causative influences, these symptoms would be expected to be highly correlated and load on a single factor. Thus, the sizeable proportion of studies reporting multiple-factor solutions supports the view that the different groups of autistic traits/symptoms are etiologically distinct.

Studying early infant precursors associated with ALTs may illuminate present understanding of the impact of etiological factors on early neurodevelopment which may, in turn, facilitate the identification of specific causative influences of ASD. Given that the different clusters of autistic traits/symptoms are phenotypically and etiologically fractionable, it is important that early precursors are studied separately in relation to each autistic dimension (Ronald & Hoekstra, 2014).

In view of the evidence, this study examined the predictive utility of early precursors in relation to later (i) social-communication (social), and (ii) non-social/behavioural (non-social) ALTs. This two-factor approach was adopted as it is in line with the dyadic symptom model of ASD in the DSM-5. Strong empirical support exists for the use of a dyadic model: a comprehensive review of 36 factor-analytic studies of autistic symptoms

revealed that a two-factor model comprising (i) social and communication-related symptoms and (ii) RRBI-related symptoms enjoyed the most empirical support relative to other multiple-factor models (Shuster et al., 2014). Moreover, the validity of this model has been found to be more robust than the triadic-symptom model employed in the DSM-IV (Mandy, Charman, & Skuse, 2012).

The following groups of early precursors were examined in relation to social and non-social ALTs in the present study—(i) prenatal, perinatal, and obstetric complications, (ii) infant temperament at 3 months, and (iii) infant social development at 12 months. These three broad categories of early precursors were of interest because they can be assessed within the first 12 months of life. Importantly, past research investigating early markers of ASD in clinical and high-risk studies has consistently found them to be associated with higher risk of later ASD. In comparison, very little research has examined the predictive utility of these precursors in relation to dimensionally measured ALTs in unselected community samples. Therefore, the present study attempted to address this gap, and in so doing, concurrently explored the extent to which findings from clinical and high-risk studies can be extended to unselected samples from the general population.

1.4. Prenatal, Perinatal, and Obstetric Complications, ASD, and ALTs

Research on the etiology of ASD has predominantly focused on the role of genes. However, the absence of perfect concordance between monozygotic twins highlights that the manifestation of autistic traits/symptoms are not solely determined by genetic influences (Bailey et al.,

1995; Folstein & Rutter, 1977; Klauck, 2006). Importantly, non-heritable risk factors have been found to have modest but significant contributions to the manifestation of autistic traits/symptoms (see Meek, Lemery-Chalfant, Jahromi, & Valiente, 2013, for a review).

Prenatal, perinatal, and obstetric complications (hereafter abbreviated as "PPOs") are a subset of non-heritable risk factors which have been implicated in a range of neurodevelopmental and psychiatric conditions (Newschaffer et al., 2007). There is substantial evidence that PPOs are associated with higher risk of later ASD (Gillberg & Gillberg, 1983; Kolevson, Gross, & Reichenberg, 2007; Piven et al., 1993; Lord et al., 1991; Sandin et al., 2013), and it is likely that the contributions of PPOs to elevated ASD risk are, in part, through interaction with genetic risk factors (Bolton et al., 1997; Yirmiya & Charman, 2010). A number of studies have found that suboptimality of the gestational environment may cause epigenetic changes in the developing fetus during pregnancy, and that these changes could affect genetic material associated with aspects of neurodevelopment linked to autistic behaviours (see Tordjman et al., 2014, for a review). Importantly, solitary PPOs do not appear to significantly increase the overall risk of later ASD (Kolevzon, Gross, & Reichenberg, 2007; Zwaigenbaum et al., 2002). The following subsections summarize empirical findings of seven PPOs in relation to ASD risk, and discuss the methodologies employed by existing studies in this area.

1.4.1. PPO risk factors associated with ASD

Advanced maternal age. Advanced maternal age is perhaps the most extensively studied of all PPOs. While most empirical studies support a positive association between maternal age and risk of ASD (Croen, Najjar, Fireman, & Grether, 2007; Hultman, Sparén, & Cnattingius, 2002), this finding is not unanimous (Reichenberg et al., 2006). It has been proposed that older expectant mothers are at greater risk of suffering from obstetric complications (Kolevzon et al., 2007; Rosenthal & Paterson-Brown, 1998) and this, in turn, raises the risk of developing ASD. It has also been speculated that the higher risk of complications may be due to genetic anomalies that arise as a consequence of aging (Ginsburg, Fokstuen, & Schinzel, 2000). However, the mechanisms through which these complications result in autistic symptoms remain unknown. Further study of this risk factor may provide insight into the biological processes that give rise to symptoms of ASD (Sandin et al., 2012).

Low birth weight. Low birth weight (LBW), often defined as birth weight of less than 2500g, is considered an indicator of possible intrauterine complications and/or problems with early fetal development (Wilcox, 2001). Evidence of the association between LBW and ASD is mixed. Some studies have reported that infants with LBW are 1.5 to 2 times more likely to receive a diagnosis of ASD in comparison with infants with normal birth weight (Eaton, Mortensen, Thomsen, & Frydenberg, 2001; Gardener, Spiegelman, & Buka, 2011; Larsson et al., 2005). However, other studies have found no such association (Juul-Dam, Townsend, & Courchesne, 2001; Stein, Weizman, Ring, & Barak, 2006). This discrepancy may be due to the significantly smaller sample sizes (N_{ASD} = 74 and N_{ASD} = 206 respectively) of the studies which did not yield significant associations, relative to those which reported

significant relationships (N_{ASD} = 698 and N_{ASD} = 3420 respectively). Thus, the lack of statistical power may have possibly prevented the detection of true associations in studies which reported a null relationship between LBW and ASD.

Prematurity. Prematurity or preterm birth, commonly defined as birth before 37 weeks of gestation, co-occurs frequently with LBW. This is not surprising since the birth weight of preterm babies is typically lower than that of their at-term counterparts (Sandin, Kolevzon, Levine, Hultman, & Reichenberg, 2013). The presence of an association between prematurity and later ASD risk is also unclear: some studies found preterm infants to be at higher risk of ASD relative to infants born at term, after controlling for covariates such as LBW (Buchmayer et al., 2009; Williams, Helmer, Duncan, Peat, & Mellis, 2008), whereas others did not (Hultman et al., 2002; Maimburg & Væth, 2006). Inconsistencies across studies could be due to inter-study differences in the gestational duration defined as "preterm" (see Guinchat et al., 2012 for a review). These inconsistent definitions, however, have highlighted the possibility that ASD risk may be positively and linearly related to extent of prematurity, as more severe prematurity seems to be related to higher risk estimates of ASD (Eaton et al., 2001; Larsson et al., 2005). It has been suggested that understanding how prematurity affects fetal and neonatal brain development may lead to a more nuanced understanding of the pathway(s) through which this risk factor contributes to the later emergence of autistic symptoms (Hofheimer, Sheinkopf, & Eyler, 2014).

Caesarean delivery. Delivery by Caesarean-section (C-section) is more prevalent in older expectant mothers and in instances of in-utero complications

such as breech presentation (Bilder, Pinborough-Zimmerman, Miller, & McMahon, 2009). This mode of delivery is also related to several other PPOs implicated in ASD, including preterm birth, LBW, and fetal hypoxia (Annibale, Hulsey, Wagner, & Southgate, 1995). Epidemiological comparisons of delivery mode (vaginal vs. C-section) have yielded mixed findings: Some studies reported that C-section delivery rates were higher in children subsequently diagnosed with ASD (Dodds et al., 2011; Glasson et al., 2004; Hultman et al., 2002) whereas other studies did not find such an association (Bilder et al., 2009; Burstyn, Sithole, & Zwaigenbaum, 2010). A key limitation of studies investigating the link between C-section delivery and ASD is that the reasons for C-section delivery are not routinely recorded (Glasson et al., 2004). This is important since a wide variety of reasons—ranging from the presence of other PPO complications to personal preference—could contribute to the choice to deliver via C-section. Thus, it is difficult to ascertain whether the association between C-section and ASD risk could be better explained by another PPO factor, or group of factors.

Prenatal smoking. It has been hypothesized that certain constituents (particularly, nicotine) in cigarette smoke have a direct adverse impact on fetal neurodevelopment (Shea & Steiner, 2008), and that these detrimental effects raise the risk of the unborn child developing ASD later in life (Newschaffer et al., 2007). Existing population-based studies provide mixed evidence on whether prenatal smoking increases risk of ASD: while Hultman and colleagues (2002) found prenatal smoking to increase the risk of ASD by 40%, other studies reported that prenatal smoking did not significantly increase risk of later ASD (Kalkbrenner et al., 2012; Lee et al., 2012). A crucial

methodological limitation of existing studies is that smoking status was determined during the first pregnancy visit. Given that around 20—40% of expectant smokers discontinue smoking during pregnancy (Cnattingius, 2004), misclassification could have hindered the detection of true associations in earlier studies. Establishing maternal smoking status later in pregnancy will likely be a more accurate method for determining smoking status.

Prenatal alcohol. Very few large studies have examined the effects of fetal alcohol exposure on the risk of developing ASD. A causal link between prenatal alcohol exposure and ASD may be possible. The neuropathology of ASD has been found to be similar to that of fetal alcohol syndrome (Ikonomidou et al., 2000). Also, social and behavioural profiles of 10-year-old children with fetal alcohol spectrum disorders (FASD) have been found to be similar to symptoms related to ASD (Stevens, Nash, Koren, & Rovet, 2012). Several single-case and small-sample studies report that the occurrence of ASD may be higher in children with FASD, providing preliminary evidence that high levels of prenatal alcohol exposure is linked with elevated ASD risk (Aronson, Hagberg, & Gillberg, 1997; Harris, MacKay, & Osborn, 1995; Mukherjee, Layton, Yacoub, & Turk, 2011). To date, only one large population-based study has investigated the risk of prenatal alcohol exposure and ASD. However, this study did not find a significant association between low-moderate alcohol consumption and ASD risk; heavy alcohol consumption was not investigated (Eliasen et al., 2010). Considered together, these findings suggest that increased risk of ASD may only be significant at higher levels of prenatal alcohol exposure. Nevertheless, it could still be possible that low-to-moderate levels of exposure are associated with higher ALTs.

Birth order. Birth order—particularly, being firstborn—has been associated with higher risk of later ASD (Bolton et al., 1997; Tsai & Stewart, 1983). Although some studies have reported that both firstborn and later-born children are at higher risk of developing ASD (Bolton et al., 1997; Piven et al., 1993), a meta-analysis by Gardener, Spiegelman and Buka (2009) found firstborn children to be 61% more likely to develop ASD later in life, compared to children born third or later. It is plausible that the higher prevalence of ASD in firstborn compared to second or later-born individuals may be an artefact of parental decisions against having more children following diagnosis (Jones & Szatmari, 1988). Studies which have investigated the association between birth order effects and the risk of ASD have yielded inconclusive findings: while some studies did find ASD risk to be higher in firstborn children (Glasson et al., 2004; Zwaigenbaum et al., 2002), others reported no effect of birth order (Hultman et al., 2002; Larsson et al., 2005). However, it was not known whether maternal age was controlled for in these studies. Turner, Pihur, and Chakravarti (2011) highlighted that this discrepancy in findings could be due to the lack of control over (i) maternal age, and (ii) degree of genetic susceptibility. In this study, being later-born was associated with increased risk of later ASD. However, the authors observed that this relationship occurred alongside a similar positive relationship between maternal age and ASD risk (Turner, Pihur, & Chakravarti, 2011). This suggests that mixed findings reported in earlier studies could have arisen partly due to the lack of control for maternal age. In addition, this study also found that first or later-born children were at higher risk in simplex families (one family member diagnosed with ASD), whereas

middle-born children were at higher risk of developing ASD in multiplex families (more than one family member with ASD). It is hoped that studying birth order in relation to individual differences in sub-threshold ALTs in an unselected community sample may provide a viable alternative for elucidating this association, since this approach eliminates the need to control for familial genetic vulnerability to ASD.

1.4.2. Methodological approaches to studying PPOs and ASD risk

Dodds and colleagues (2011) outlined two general approaches which have so far been employed in efforts to elucidate the relationship between PPOs and ASD. One approach adopted by many studies (i.e. Glasson et al., 2004; Hultman et al., 2002; Juul-Dam, Townsend, & Courchesne, 2001; Larsson et al., 2005; Maimburg & Væth, 2006; Piven et al., 1993) involves examining each PPO individually in relation to ASD risk by obtaining Odds Ratios (ORs) or Relative Risk (RR) ratios for each risk factor. While this method allows the identification of unique associations for each factor, the accuracy of individual risk estimates is compromised when the PPO of interest is known to co-occur frequently with other complications (Dodds et al., 2011). For example, LBW and prematurity have been found to be highly correlated (Sandin et al., 2013). Older mothers are also more likely to undergo Caesarean delivery (Bilder et al., 2009). Moreover, this method does not account for the cumulative effects of multiple PPOs on ASD risk (Dodds et al., 2011).

The second approach involves evaluating the overall influence of PPO-related complications on the risk of ASD (Gillberg & Gillberg, 1983; Bryson, Smith, & Eastwood, 1988; Bolton et al., 1994). Overall obstetric severity is

estimated by deriving a composite 'optimality' or 'suboptimality' score, where having a greater number of PPOs results in lower 'optimality' or higher 'suboptimality' scores (Dodds et al., 2011). Studies adopting this approach have found that individuals with ASD consistently obtain lower optimality scores (Bryson et al., 1988; Zwaigenbaum et al., 2002) or higher suboptimality scores (Bolton et al., 1997; Lord, Mulloy, Wendelboe, & Schopler, 1991; Stein, Weizman, Ring, & Barak, 2006) relative to non-ASD controls. These findings strongly support an inverse relationship between obstetric optimality and risk of ASD. This method is suitable for smaller sample sizes and when multiple PPOs are found to be highly correlated with each other (Sandin et al, 2013). However, it is limited in that each factor is treated with equal importance, and thus, does not provide much information regarding the unique associations between each PPO and ASD risk (Dodds et al., 2011).

1.4.3. Might PPOs explain autistic trait variability in the general population?

To date, only one study has explored the association between prenatal and neonatal risk factors, and later ALTs in a sample of 13690 twins (Ronald, Happé, Dworzynski, Bolton, & Plomin, 2010). This study found that prenatal and neonatal complications collectively accounted for a modest but statistically significant amount (2—5%) of the variance in ALTs at 7-8 years of age; weak relationships between risk factors and ALTs were observed in both the normal and quantitative extreme ranges (Ronald et al., 2010). However, prenatal and neonatal data were retrospectively obtained from

mothers via self-report only when their infants were around 18 months of age. Hence, the accuracy of the data may have been somewhat compromised.

The present study aimed to build on these earlier findings by prospectively investigating whether PPO risk factors are predictive of dimensionally measured ALTs at 18 months in a general population sample. This investigation extends existing research by exploring whether PPOs are reliable indicators of ALTs in toddlerhood. Furthermore, previous research examining the role of PPOs in ASD has been based on clinical populations. In this study, birth and obstetric information was prospectively collected during the middle pregnancy, as well as at the time of delivery, so as to minimize inaccuracies that may arise from retrospectively gathered information due to memory-related distortions or biases.

1.5. Infant Temperament

1.5.1. Temperament and its association with child psychopathology.

Temperament has been defined by Thomas and Chess (1977) as genetically determined individual differences in behavioural tendencies. According to this definition, a child's disposition to react in a particular way to a given environmental stimulus is also based on heritable influences and biological makeup. Thomas and Chess identified nine dimensions of behaviour which they considered to be related to psychological development (summarized in Table 1).

Table 1.

Definition of the nine dimensions of temperament proposed by Thomas and Chess

Dimension	Description
Activity	The level, tempo, and frequency with which a motor component is present in a child's functioning.
Rhythmicity	The degree of regularity of repetitive biological functions.
Distractibility	The effectiveness of extraneous environmental stimuli in interfering with, or in altering the direction of, the ongoing behaviour.
Approach	The child's initial reaction to any new stimulus, be it food, people, places, toys, or procedures.
Adaptability	The ease or difficulty which the initial pattern of response can be modified in the direction desired by the parents or others.
Persistence	The child's maintaining an activity in the face of obstacles to its continuation.
Threshold	The level of extrinsic stimulation that is necessary to evoke a discernable response.
Intensity	The energy content of the response, irrespective of whether it is positive or negative.
Mood	The amount of pleasant, joyful, friendly behaviour as contrasted with unpleasant, crying, unfriendly behaviour.

[a] Descriptions quoted verbatim from Thomas et al. (1968), pp. 19-24.

Another well-accepted theoretical definition of temperament was conceptualized by Rothbart and Bates (1998), who defined temperament as biologically determined individual differences in response intensity and self-control. They proposed that temperament consists of three broad dimensions of behaviours: (i) surgency or extraversion—sociability, activity level, and behaviours displaying positive affect (Rothbart, Derryberry, & Hershey, 2000); (ii) negative affectivity—the tendency to experience negative emotions and mood states such as fear, anxiety, and anger (Rothbart, Chew, & Gartstein, 2001); and (iii) effortful control—the capacity to restrain emotions

and behaviour and manage attentional resources purposefully (Rothbart & Putnam, 2002).

A number of temperament measures have been constructed following Rothbart and Bates' theoretical model, including the Infant Behaviour Questionnaire-Revised (IBQ; Gartstein & Rothbart, 2003) and the Early Childhood Behaviour Questionnaire (ECBQ; Putnam, Gartstein, & Rothbart, 2006). On the other hand, the Carey Temperament scales (CTS; Carey & McDevitt, 1995)—a series of measures designed to assess temperament from one month to 12 years of age—have been developed based on Thomas and Chess' framework.

Thomas and Chess' model of temperament was adopted in this study for several reasons. Firstly, this theoretical model was developed through the landmark New York Longitudinal Study (NYLS; Thomas, Chess, Birch, Hertzig & Korn, 1963)—a large population cohort study designed for the purpose of identifying early behavioural patterns predictive of behavioural problems later in childhood (Rothbart, Chew & Gartstein, 2001). Secondly, the NYLS assessed temperament in infants as young as 2-6 months of age. Given that the present study is concerned with examining whether infant temperament as early as 3 months is predictive of later ALTs, this model of temperament is therefore more relevant in relation to the purposes of this study. Finally, studies which have longitudinally investigated the relationship between early infant temperament and later ALTs (e.g. Bolton, Golding, Emond, & Steer, 2012; del Rosario, Gillespie-Lynch, Johnson, Sigman, & Hutman, 2014) have used scales from the CTS to measure temperament. Thus,

to facilitate comparison with these studies, the Thomas and Chess' (1977) approach and the CTS were employed in the present study.

1.5.2. Temperament and its association with ASD/ALTs

Studying infant temperament as a potential early marker of ALTs may lead to the identification of endophenotypes—behavioural precursors of future psychopathological symptoms which are associated with genetic causes (Miller & Rockstroh, 2013)—relevant to ASD. This could in turn provide insight into the neurobiological basis of ASD and, consequently, shed light on how the etiological factors underlying ASD influence early life neurobiological development (Garon et al., 2009). Since infants' behavioural tendencies are increasingly shaped by environmental influences during later childhood (Mervielde & De Pauw, 2012), the present study assessed temperament during early infancy as this would provide a more accurate estimate of an individual's genetically determined behavioural tendencies.

Thus far, most of the research which has studied the association between temperament and ASD has focused on comparing temperament profiles of (i) children diagnosed with ASD vs. typically developing children, or children with other delays, (ii) high-risk infant siblings who were later diagnosed with ASD (sibs-ASD) vs. high-risk infant siblings who did not receive a later diagnosis of ASD (sibs-TD). To date, only one large study (Bolton, Golding, Emond, & Steer, 2012) has attempted to assess the predictive utility of early temperament on later autistic traits in an unselected community sample. To facilitate easier comparison of findings between studies, only empirical studies which employed the CTS as a measure of

temperament are discussed. Hepburn and Stone (2006) compared differences in the temperament profiles of 3-8 year old children with ASD (N = 110) against normative data reported by McDevitt and Carey (1978) that was based on a sample of 350 TD children. In comparison with data from the normative sample, Hepburn and Stone's sample of children with ASD scored at least one standard deviation above the mean on the Adaptability and Persistence dimension, and one standard deviation below the mean on the Threshold dimension. These findings showed that children with ASD were significantly less adaptable, less persistent, and less responsive (i.e. higher threshold of responsiveness) to external stimuli, compared to their typically developing counterparts (Hepburn & Stone, 2006).

More recently, Brock and colleagues (2012) compared the temperament profiles of 3-7 year-old children with ASD (n = 54) and developmental delay (DD; n = 33) against the same normative sample (McDevitt & Carey, 1978). They found significant differences between their ASD sample and the normative sample on 8 of the 9 temperament dimensions—children with ASD were more active, less rhythmic, less approaching, less adaptable, less intense, less persistent, less distractible, and less responsive to environmental stimuli as compared to TD children (all ps < .001). Relative to children with DD, children with ASD were less approaching (p = .018) and less distractible (p = .004). The findings suggested that children with ASD could be distinguished from TD and DD children based on their temperament profiles.

Findings from these studies, which were based on clinical samples, have shown that children diagnosed with ASD may be distinguished from

healthy children and children with developmental delays based on their temperament profiles. However, the between-group comparisons were based on previously published normative data rather than a prospectively recruited sample of age-matched controls. Moreover, both studies investigated temperament differences at 3 years of age and later; children who do develop ASD would likely have already received a diagnosis by that age (Chakrabarti & Fombonne, 2001). Studying temperament-related differences that may arise during infancy would therefore be of greater clinical value in terms of facilitating early identification of children who may be at risk of developing ASD or high levels of ALTs.

A recent prospective longitudinal study of high-risk infant siblings by del Rosario and colleagues (2014) examined whether early differences in temperament trajectories could discriminate high-risk siblings who develop ASD later in life from those who do not. They prospectively tracked the temperament trajectories of 43 high-risk infant siblings from 6 to 36 months of age by inviting parents to complete the CTS at 6-month intervals. At 6 and 12 months of age, high-risk infants later diagnosed with ASD (sibs-ASD; $n = 16$) exhibited greater adaptability than their counterparts who were not subsequently diagnosed (sibs-TD; $n = 27$), with moderate to large effect size difference (Cohen's $d = 0.70$—1.38). However, this trend was reversed at 24 months (i.e. sibs-ASD now exhibited lower levels of adaptability than sibs-TD; $d = 0.40$) and became more pronounced at 36 months ($d = 1.13$). A similar pattern was observed for approach: sibs-ASD displayed higher levels of approach-related behaviours than sibs-TD at 6 months ($d = 0.95$), but lower levels of approach-related behaviours than the latter at 24 months ($d = 0.63$)

and 36 months of age (d = 1.38). The findings suggest that temperament may be useful in identifying high-risk infants who go on to develop ASD. However, generalizability of these findings was constrained by the limited sample size (del Rosario et al., 2014)—a common limitation of research efforts investigating the predictive utility of infant temperament on ASD risk using case-control and high-risk samples (Garon et al., 2009; Zwaigenbaum et al., 2005).

Bolton and colleagues (2012) were the first to examine infant temperament, among many other early precursors, as a predictor of dimensionally measured ALTs in the general population. They prospectively and longitudinally studied 14387 children, all of whom were participants of the Avon Longitudinal Study of Parents and Children (ALSPAC). Temperament was assessed at 6 and 24 months, and examined in relation to ALTs at 30 months. ALTs were assessed using a composite measure autistic-like behaviours which drew from information obtained from several measures—such as caregiver-report questionnaires, observational, and standardized assessments which contained items related to autistic-like behaviours (Bolton et al., 2012). The authors found that lower levels of activity, rhythmicity, approachability, adaptability, persistence, intensity, more negative mood, and higher distractibility and threshold of responsiveness at 6 months were associated with more ALTs at 30 months. A largely similar temperament profile at 24 months predicted more ALTs at 30 months, except that higher (rather than lower) levels of activity and intensity were now associated with higher levels of later ALTs (Bolton et al., 2012). A key limitation of this study, however, was that the composite measure of ALTs

employed was partly derived from measures of some of the predictors of interest. Thus, the authors cautioned that scores obtained on their measure of ALTs may be partially confounded (Bolton et al., 2012).

The work of del Rosario et al. (2014) and Bolton et al. (2012) highlighted that temperament measured as early as 6 months in infancy may be predictive of higher levels of later ALTs. However, one way in which they differed was that greater and lower adaptability at 6 months were respectively implicated in . These differences could be because both studies utilized different samples and had starkly different sample sizes.

1.5.3. Might infant temperament as early as 3 months be predictive of variability in social/non-social ALTs at 18 months?

There is reasonably strong evidence that early temperament is likely associated with the later emergence of ASD/ALTs, and that infant temperament could be a useful predictor of the emergence of later autistic traits or symptoms in children. However, no study has examined the predictive utility of infant temperament separately in relation to social and non-social ALTs in toddlerhood, using a standardized and validated measure of ALTs. Furthermore, no study has explored whether different temperament dimensions may be associated with different clusters of ALTs. It is possible that such differences may exist: for example, adaptability and persistence may be more strongly associated with non-social/behavioural ALTs, which are characterized by preference for routine and insistence on sameness. On the other hand, approach and threshold may respectively reflect the degree of

preference for aloneness or responsiveness to social stimuli, and thus might possibly be related to social communication ALTs.

The present study examined whether earlier findings pertaining to the association between temperament and ASD/ALTs could be extended to an unselected sample from a different ethnic population, using a standardized, validated measure of ALTs. Importantly, it aimed to further existing knowledge by assessing the relationship between temperament (3 months) and ALTs (18 months) at earlier time-points compared to all previous studies, and also explored whether different temperament dimensions might be predictive of later social and/or non-social ALTs.

1.6. Infant Social Development in the first 12 months

Social competence has been defined by Cavall (1990) as a multi-dimensional construct comprising of (i) basic social skills essential for adaptive social functioning, (ii) the ability to interact positively and purposefully with the social environment, and (iii) social adjustment—the ability to attain developmentally appropriate goals. Development of social competencies is a prerequisite for successful social and academic functioning in children (Gest, Sesma, Masten, & Tellegen, 2006). In contrast, delays in the attainment of these competencies predispose the individual to a wide range of social and behavioural difficulties (Yeates et al., 2007).

The present study focused on three aspects of infant social development that have been implicated in ASD: (i) gesture use, (ii) imitation/play skills, and (iii) empathy. Although deficits in these abilities are not unique to ASD, delays in their development have been found, in numerous

retrospective and prospective studies, to precede later emergence of ASD (Baird et al., 2000; De Giacomo & Fombonne, 1998; Hoshino et al., 1987; Ozonoff et al., 2010; Rogers & DiLalla, 1990; Swinkels et al., 2006).

1.6.1. Gestures, ASD, and ALTs

The development of verbal communication in infants has been observed to be contingent on the successful development of more rudimentary non-verbal communication abilities, such as the use of gestures (Fenson et al., 1994). Gestures allow for symbolic sharing of a child's thoughts, feelings, and desires with other social agents (Mitchell et al., 2006) and facilitate the expression of needs and making of requests (Landa, 2007). In typically developing infants, gesture development forms the basis for more complex forms of non-verbal communication, such as responding to joint attention (RJA) and initiating joint attention (IJA) (Bakeman & Adamson, 1984; Sullivan et al., 2007).

Retrospective studies of clinical samples of children with ASD have found that delays in the recognition and use of gestures are noticeable in children with ASD as early as 12 months of age. Common signs include reduced pointing and showing, gesture imitation, and engagement in joint attention (Mars, Mauk, & Dowrick, 1998; Werner & Dawson, 2005). Analyses of home-videos of children diagnosed with ASD show that poorer early use of gestures is associated with less frequent orientation to name (Osterling & Dawson, 1994; Werner, Dawson, Osterling, & Dinno, 2000) and poorer comprehension of phrase speech (Thal & Bates, 1988). Thus, deficits in gesture use appear to contribute to some of the social-communication

impairments characteristic of ASD. However, retrospective home-video studies do not allow for the systematic comparison of developmental trajectories of children who present with elevated levels of later autistic traits/symptoms versus those who do not (Mitchell et al., 2006).

Prospective longitudinal studies of high-risk infant siblings which followed the development of these children from infancy to childhood have found that deficits in nonverbal communication within the first two years of life are associated with higher levels of social-communication related ALTs, as well as higher risk of developing ASD in early childhood. Mitchell and colleagues (2006) followed the early communication and language development of high-risk infant siblings (n = 97) and low-risk controls (n = 49) from 12 to 18 months of age using the *MacArthur Communicative Development Inventories* (CDI; Fenson et al., 1994). They discovered that infant siblings later diagnosed with ASD (n = 15) used gestures less frequently at 12 months. Importantly, high-risk siblings who did not develop ASD exhibited reduced gesture use compared to low-risk controls at 18 months, after accounting for language delays (Mitchell et al., 2006). These results suggest that delays in gesture development may predict higher levels of ALTs, regardless of a diagnosis of ASD.

Another prospective study by Ibanez, Grantz and Messinger (2013) examined the extent to which three forms of early referential communication—responding to joint attention (RJA), initiating (IJA), and initiating behavioural requests (IBR)—predicted the severity of later autistic symptoms in a sample of 40 high-risk infant siblings and 21 low-risk controls. They charted the developmental trajectories of these early communication

behaviours at 2 to 3 month intervals, from 8 to 18 months of age. Autistic symptom severity was assessed at 30 months of age using the calibrated severity score of the ADOS. This study found that, relative to the controls, high-risk siblings displayed lower overall levels of RJA and IJA, and a lower overall rate of IBR development. When a separate analysis was conducted excluding 10 high-risk infants who were later diagnosed with ASD, RJA development in the high-risk group was still found to be significantly poorer than in the low-risk group. Furthermore, it was found that baseline IJA and the rate of IBR development predicted autistic trait severity within the high-risk group at 30 months. The findings of this study resonate with that obtained by Mitchell and colleagues (2006), suggesting that the ability to use gestures in early social communication behaviours is a potential predictor of later ALTs regardless of a diagnosis of ASD.

Few studies have explored whether the relationship between infant gesture development and later diagnosis of ASD is also found across the whole range of ALTs. To date, only one population-based study has reported that lower levels gesture use at 15 months predicted higher levels of ALTs at 30 months (Bolton et al., 2012). The present study aimed to extend these findings by investigating whether limited gesture use at 12 months of age may be predictive of more ALTs at as early as 18 months.

1.6.2. Imitation, Play, ASD, and ALTs

The lack of imitation and spontaneous pretend play behaviours have consistently been identified as early precursors of ASD in studies conducted on clinical samples. Retrospective analyses of home-videos consistently report

that children later diagnosed with ASD exhibit reduced rates of imitation and play-related behaviours at around the first year of life (Adrien et al., 1993; Baranek, 1999; Osterling & Dawson, 1994). These findings are in line with those from prospective studies, which compared the imitation and play behaviours of children with ASD with that of children with other developmental conditions. Charman and colleagues (1997) reported that deficits in imitation were more profound in 20-month-old children with autism (n = 12) than in children of the same age who had developmental delays (n = 44). Rogers, Hepburn, Stackhouse and Wehner (2003) compared the imitation abilities of 21-month to 50-month-old children with ASD (n = 24) with those of children with Fragile X syndrome (n = 18), other development disorders (n = 20), and healthy controls (n = 15), with all groups having relatively similar mental ages. They reported that children with ASD were found to perform more poorly across three out of four imitation-related tasks, relative to all other groups. In addition, the degree of impairment in imitation skills within the ASD group was positively associated with autistic severity (r = .49—.73) with moderate to large effect sizes (Rogers et al., 2003).

Prospective studies of high-risk infant siblings reveal that poor development of imitation and pretend play are likely associated with the presence of higher levels of ALTs. Zwaigenbaum and colleagues (2005) reported that high-risk siblings who later developed ASD could be distinguished from high-risk siblings who did not, as the former displayed significantly less imitative behaviours and engagement in pretend play at 12 months of age compared to the latter. These differences also appear to extend to the non-extreme ranges of the autistic severity continuum. Christensen and

colleagues (2010) compared the play behaviours of 17 high-risk infant siblings with 12 high-risk siblings with non ASD-related delays and 19 TD controls using a free-play task. High-risk infants showed fewer functional play behaviours than infants from the other groups at 18 months of age, suggesting that atypical play behaviours may be an indicator of social difficulties specific to ALTs, rather than other types of developmental delay. At this point, it is important to note that the sample sizes of the earlier described studies were small, hence limiting the conclusiveness of the findings discussed.

Few studies have examined whether imitation and play-related behaviours may be predictive of later ALTs in community samples. Drawing from an investigation of a subsample of approximately 6000 children from their ALSPAC sample, Bolton and colleagues (2012) found that lower levels of imitation and play-related behaviours as early as 6 months predicted higher ALTs at 30 months. However, this finding may be obscured due to confounding; some of the items from the measures of imitation and play behaviours were concurrently used by the authors in the computation of participants' overall autistic trait score (Bolton et al., 2012).

The present study aimed to extend previous findings by exploring whether imitation and play-related behaviours are predictive of ALTs at as early as 18 months of age. In addition, it addressed the limitation highlighted in Bolton and colleagues' study by assessing ALTs using a dimensional measure of ALTs with reasonable psychometric properties.

1.6.3. Empathy, ASD, and ALTs

Empathy refers to the ability to interpret another person's mental and emotional states, and respond in a socially appropriate manner (Davis, 1994). Deficits in empathy have been found to characterize a number of psychiatric conditions, including schizophrenia (Bora, Gökçen, & Veznedaroglu, 2008), psychopathy (Mealey & Kinner, 2002), as well as ASD (Schroeder, Desrocher, Bebko, & Cappadocia, 2010).

Retrospective and prospective studies of infants diagnosed with ASD have reported that infants diagnosed with ASD tend to display reduced interest in social interaction (Goldberg et al., 2005), less social smiling (Adrien et al., 1993), and deficits in exhibiting socially appropriate affective responses in experimental tasks designed to elicit empathic behaviour (Sigman, Kasari, Kwon, & Yirmiya, 1992). These empathy-related deficits have been reported to be present in infants with ASD by about 20 months of age (Charman et al., 1997). However, no published study has investigated whether empathy might be predictive of dimensionally measured ALTs in community samples of children.

Research on community samples of adults has found that empathy may be a predictor of ALTs in the general population. A study by Wheelwright and colleagues (2006) reported that scores on the Empathy Quotient (EQ; Baron-Cohen & Wheelwright, 2004)—a self-report instrument purported to measure an individual's drive to empathize—were significantly predictive of scores on a self-report quantitative measure of ALTs—the Autism Spectrum Quotient (AQ; Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). These findings have also been replicated in an Asian sample (Wakabayashi et al.,

2007), providing cross-cultural support for the association between empathy and autistic trait variability in non-clinical populations.

At present, no study has explored whether empathy during infancy may be predictive of dimensionally-measured autistic traits in toddlerhood. This could be due to the inherent challenges of attempting to assess empathy within the first year of life, since it is difficult to determine whether the absence of an empathic response is due to the presence of autistic traits/symptoms, or a more generic lack of perceptual understanding of displays of affect (Auyeung et al., 2009). Nevertheless, given that children with ASD under 2 years of age have been reported to have difficulty displaying socially appropriate affective responses or emotional mimicry in response to an emotional reaction exhibited by social agents in experimental settings (Hobson, 1993; Sigman et al., 1992), it may be worth investigating whether empathy in infancy predicts later ALTs. Hence, the present study explored whether empathy at 12 months predicted the variability of ALTs in a sample of 18-month-old toddlers from the general population.

1.6.4. Might infant social development at 12 months be predictive of variability in social/non-social ALTs at 18 months?

In light of recent findings that social and non-social ALTs are phenotypically distinct (Shuster, Perry, Bebko, & Toplak, 2014) and underpinned by independent sets of etiological influences (Ronald et al., 2006a), it is important to investigate whether early precursors linked with ASD may be specifically related to and predictive of social ALTs. Given that gestures, imitation, play, and empathy are all early competencies associated

with social and communication development in childhood, it is hypothesized in this study that they will be more strongly associated with social-communication, rather than with non-social/behavioural, ALTs. Exploring this possibility is important for clarifying the neurodevelopmental link between early individual differences in social-communication development and later ALT variability. Thus, the present study assessed the predictive utility of each of the earlier discussed variables separately in relation to social and non-social ALTs.

1.7. The Present Study: Rationale, Aims, Research Questions, and Hypotheses

Instead of the categorical approach employed by many previous studies which have investigated early markers of ASD, this study adopted a dimensional approach to measuring and understanding potential early predictors of ALTs. The dimensional approach is aligned with a substantial body of evidence demonstrating (i) etiological and phenotypic similarity throughout the autism spectrum, and the (ii) normal distribution of ALTs in the general population. Studying ALTs in unselected community samples also eliminates the drawbacks of the methodological challenges common in clinical case-control studies and high-risk sibling studies, including limited statistical power due to very small sample sizes (typically <50 and in many cases <30), studying only one or two predictors, and investing large amounts of resources for the surveillance of a large number of individuals, of which only a small fraction (typically 5—25 %) will be diagnosed with ASD and thus be eligible for study participation.

While numerous early precursors have been implicated in the manifestation of later ASD/ALTs, how they contribute to the variability of specific clusters of ALTs over time has not yet been explored. Such an investigation is important in view of strong empirical evidence suggesting that different clusters of ALTs are etiologically fractionable and therefore may be associated with different early precursors. Furthermore, existing studies have typically focused either on a single precursor or a small number of precursors, without considering how their effect on ALTs might relate to other early markers. Studying the unique contributions of a wider range of early precursors on ALTs may provide better insight into the relative importance of different predictors in their influence on the development of autistic-like behaviours. This could then inform more focused future investigations of the neurodevelopmental processes that may be affected by etiological factors in those with ASD or at the extreme ends of the continuum of autistic traits.

This study is the first to prospectively investigate the associations between early precursors within the first year of life and ALTs at 18-months—earlier than all the studies reviewed. It is also the first to examine the predictive utility of early precursors separately in relation to social and non-social ALTs, in light of strong evidence suggesting that the different ALT clusters are etiologically fractionable. Finally, this is first study to examine the predictive utility of early precursors with later ALTs in an unselected sample of toddlers in Asia. To the best of the author's knowledge, no study has examined the role of early precursors on later ALTs in Asia. However, such a cross-cultural investigation is important because cultural factors have been implicated in differences relating to the interpretation and measurement of

ALT in adults from Western compared to Eastern cultures (Freeth, Sheppard, Ramachandran, & Milne, 2013). Hence, this study also explored the degree to which (i) the relationships between early infancy precursors and later ALTs, and (ii) the hypothesis that different ALT clusters are etiologically fractionable, both of which have been based in Western samples, can be generalized to a sample of Asian toddlers from the general population.

The present study had two main aims:

(1) To investigate the relationship between, and predictive value of, early development precursors (namely, PPO risk factors, infant temperament at 3 months, infant social development at 12 months—gestures, imitation/play, and empathy) and social and non-social ALTs at 18 months.

(2) To examine whether precursors found to be associated with and to predict social ALTs are different than those found to be associated with non-social ALTs.

The following specific research questions and corresponding hypotheses were investigated:

Research Question 1: Do the early precursors of ASD/autistic traits identified in this study within the first 12 months of life contribute uniquely to predicting ALTs at 18 months?

It was hypothesized that each of the following categories of early variables—(i) PPO risk factors (gestation), (ii) infant temperament (3 months) and (iii) infant gestures, imitation and empathy (12 months) will be significantly correlated with 18-month Q-CHAT social and/or non-social factor scores, and that each will independently and

significantly explain the variance in 18-month Q-CHAT social and/or non-social factor scores (Hypothesis 1).

Research Question 2: Are there different early precursors for social ALTs compared to non-social ALTs?

It was hypothesized that infant social development at 12 months would be significantly associated with and predict 18-month Q-CHAT social factor scores only (Hypothesis 2).

Currently, as no study has yet examined the contribution of PPOs and infant temperament as predictors of social and non-social ALTs separately, there is no evidence upon which to make specific hypotheses as to whether these early variables will differentially predict social and non-social ALTs, thus analyses regarding these variables were exploratory.

CHAPTER 2

METHOD

2.1. Participants

2.1.1. Recruitment

Participants for the present study were drawn from an unselected, nationally representative subset of expectant mothers recruited for a large ongoing nationwide epigenetic medical research study in Singapore (GUSTO—Growing Up in Singapore Towards healthy Outcomes). GUSTO is a prospective, longitudinal study aimed primarily at identifying early life epigenetic and pregnancy-related risk factors for a wide variety of medical and genetic/biological conditions. The overarching goals of the GUSTO study are to advance our understanding of the influence of genetic and environmental risk factors in early childhood development, and the discovery of viable strategies for prevention and early management (Soh et al., 2013).

Mothers for the GUSTO study were recruited from two leading hospitals in child delivery and neonatal care, KK Women's and Children's Hospital (KKH) and National University Hospital (NUH), during the first trimester of their pregnancy, between June 2009 and September 2010. The sample recruited from KKH and NUH is likely to be representative of the general population in Singapore, as these hospitals collectively deliver approximately half of all babies born in Singapore annually[1] (Ministry of Health Singapore, 2014). GUSTO mothers and their infants (N = 1152) were

[1] Between 1st Feb 2013 to 31st Jan 2014, public hospitals performed 48% of all normal (vaginal) deliveries, and of these, 71% and 20% of these deliveries performed at KKH and NUH respectively (Ministry of Health Singapore, 2014).

subsequently followed up as part of the GUSTO study at regular intervals starting from 12 weeks of gestation, up till the child reached 36 months of age.

2.1.2. GUSTO Inclusion and exclusion criteria

Eligibility for recruitment into the GUSTO study was based on the following inclusion criteria: (i) both parents had to be either Singapore citizens or Singapore Permanent Residents; (ii) mothers were at least 18 years of age at the time of recruitment; (iii) both parents and grandparents were racially/ethnically homogeneous; and (iv) parents intended to deliver in KKH or NUH and reside in Singapore for the next five years.

Mothers were excluded from the study if they (i) had been diagnosed with serious medical conditions such as cancer or type I diabetes, (ii) were receiving chemotherapy or psychotropic medication, (iii) conceived via in-vitro fertilisation (IVF), or (iv) if pregnancy ended in miscarriage.

2.1.3. Participant characteristics

A total of 1152 mothers and their infants were recruited for the large GUSTO study. Of these, a subsample of 613 participants were specifically recruited for the GUSTO Neurodevelopment domain—these participants were followed up more extensively with a focus on more comprehensive assessment of the children's neuropsychological, cognitive, behavioural, social, and emotional development. In order to address the research questions of the principal investigators of the Neurodevelopment domain, and in consideration of the logistical and budget constraints of conducting comprehensive testing on all 1152 GUSTO participants, participants were prioritized for inclusion in

the GUSTO Neurodevelopment domain if magnetic resonance imaging (MRI) data after birth were available, if they were non-Chinese, or if they were exclusively breast- or bottle-fed, in line with the main research questions and aims of the principal investigators of the GUSTO Neurodevelopment Domain.

Of the 613 caregivers recruited by the GUSTO Neurodevelopment domain, 368 (60.0%) satisfactorily completed and returned the main measure of ALTs employed in the present study (the Q-CHAT; see *Measures*) for the 18-month follow up of their infants. As ALTs at 18 months of age was the main outcome variable for the present study, participants who did not complete the Q-CHAT at this time-point were excluded. Some parents did not complete all other measures of interest to this study. Hence, the size of the resultant sample of 368 participants was further reduced based on whether parents satisfactorily completed the other measures of interest—namely, PPO information inventories, Carey's Temperament Scale (CTS) ratings at 3 months, the Singapore English Communicative Development Inventories (*SECDI;* Tan, Liu, Affendi & Chen, 2006) at 12 months, and the Infant-Toddler Social and Emotional Assessment (ITSEA; Briggs-Gowan & Carter, 1998) at 12 months. *Figure 1* shows the breakdown of the original GUSTO sample into the different subsamples utilized for this study.

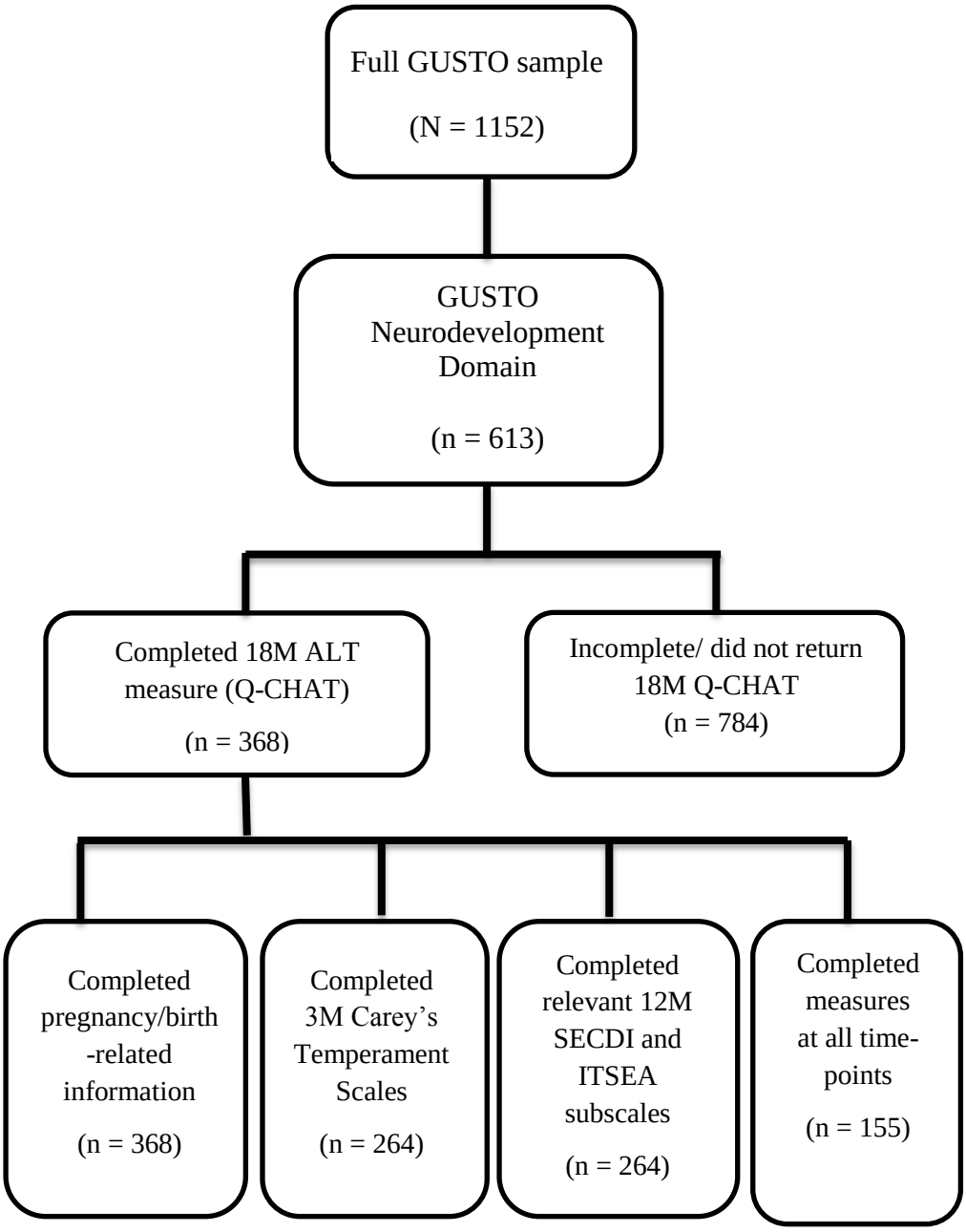

Figure 1. *Breakdown of full GUSTO sample into the available subsamples employed for analyses in the present study.*

Table 2 displays the demographic characteristics of the subsamples relevant to the present study. One sample *t*-tests and chi-square goodness-of-fit tests were used to identify any differences in characteristics between the subsamples of interest and the main GUSTO sample. All subsamples had somewhat more Malay participants and fewer Indian participants than the main GUSTO sample. In addition, some subsamples differed significantly from the full GUSTO sample in birth order, maternal education, or household income. However, since the effect sizes for these observed demographic differences were small (all $V < .20$), the subsamples are, on the whole, likely to be approximately demographically representative of the main GUSTO sample.

Table 2.

Comparison of demographic characteristics for full GUSTO sample and each of the subsamples involved in the main analyses

	Full GUSTO sample	**Completed 18M Q-CHAT and PPO questionnaire**	**Completed 18M Q-CHAT and 3M CTS**	**Completed 18M Q-CHAT and 12M ITSEA Imitation/Play and Empathy subscales**	**Completed all measures**
	N (%) or Mean (SD)	**N (%) or Mean (SD)**	**N (%) or Mean (SD)**	**N(%) or Mean (SD)**	**N(%) or Mean (SD)**
Sample size (N)	1152	368	264	209	155
Gender					
Male	571 (49.6%)	200 (54.3%)	140 (53.0%)	111 (53.1%)	80 (51.6%)
Female	517 (44.9%)	168 (45.7%)	124 (47.0%)	98 (46.9%)	75 (48.4%)
Missing	64 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		$\chi 2(1) = .51$, $p = .47$ $V = 0.04$	$\chi 2(1) = .03$, $p = .86$ $V = 0.01$	$\chi 2(1) = .03$, $p = .86$ $V = 0.01$	$\chi 2(1) = .05$, $p = .83$ $V = 0.02$
Ethnicity					
Chinese	623 (54.1%)	195 (53.0%)	144 (54.5%)	117 (56.0%)	87 (56.1%)
Malay	314 (27.3%)	119 (32.3%)	88 (33.3%)	70 (33.5%)	54 (34.8%)
Indian	208 (18.1%)	53 (14.4%)	31 (11.7%)	22 (10.5%)	14 (9.0%)
Other/Missing	7 (0.6%)	1 (0.3%)	1 (0.4%)	0 (0.0%)	0 (0.0%)
		$\chi 2(3) = 6.97$, $p = .07$ $V = 0.08$	**$\chi 2(3) = 9.64$, $p = .022$ $V = 0.11$**	**$\chi 2(2) = 9.62$, $p = .008$ $V = 0.12$**	**$\chi 2(2) = 10.3$, $p = .006$ $V = 0.18$**
Birth Order					
First	467 (40.5%)	157 (42.7%)	118 (44.7%)	94 (45.0%)	73 (47.1%)
Second	383 (33.2%)	111 (30.2%)	77 (29.2%)	71 (34.0%)	49 (31.6%)
Third or later	235 (20.4%)	98 (26.6%)	68 (25.8%)	44 (21.1%)	33 (21.3%)
Missing	67 (5.8%)	2 (0.5%)	1 (0.4%)	0 (0.0%)	0 (0.0%)
		$\chi 2(3) = 26.1$, $p < .001$ $V = 0.15$	**$\chi 2(3) = 19.6$ $p < .001$ $V = 0.16$**	$\chi 2(2) = 0.32$, $p = .85$ $V = 0.03$	$\chi 2(2) = 1.20$, $p = .55$ $V = 0.06$

	Full GUSTO sample	Completed 18M Q-CHAT and PPO questionnaire	Completed 18M Q-CHAT and 3M CTS	Completed 18M Q-CHAT and 12M ITSEA Imitation/Play and Empathy subscales	Completed all measures
	N (%) or Mean (SD)	**N (%) or Mean (SD)**	**N (%) or Mean (SD)**	**N(%) or Mean (SD)**	**N(%) or Mean (SD)**
Mother's age					
	30.4 (5.2)	30.3 (5.2) $t(367) = -.27$ $p = .79$ $d = -0.02$	30.4 (5.3) $t(263) = -.02$ $p = .99$ $d = 0.00$	30.2 (5.2) $t(208) = -.53$ $p = .60$ $d = -0.04$	30.2 (5.3) $t(154) = -.44$ $p = .66$ $d = -0.04$
Mother's highest education					
None/Primary	56 (4.9%)	14 (3.8%)	9 (3.4%)	5 (2.4%)	2 (1.3%)
Secondary/ITE	416 (36.1%)	122 (33.2%)	82 (31.1%)	63 (30.1%)	45 (29.0%)
Pre-U/Diploma	279 (24.2%)	100 (27.2%)	70 (26.5%)	61 (29.2%)	44 (28.4%)
University	348 (30.2%)	114 (31.0%)	89 (33.7%)	71 (34.0%)	57 (36.8%)
Missing	53 (4.6%)	18 (4.9%)	14 (5.3%)	9 (4.3%)	7 (4.5%)
		$\chi 2(4) = 3.20$, $p = .52$ $V = 0.05$	$\chi 2(4) = 4.94$, $p = .29$ $V = 0.07$	$\chi 2(4) = 7.83$, $p = .10$ $V = 0.10$	**$\chi 2(4) = 9.54$, $p = .05$ $V = 0.12$**
Monthly household income					
0-1999	177 (15.4%)	53 (14.4%)	40 (15.2%)	27 (12.9%)	18 (11.6%)
2000-3999	338 (29.3%)	106 (28.8%)	65 (24.6%)	47 (22.5%)	29 (18.7%)
4000-5999	271 (23.5%)	85 (23.1%)	61 (23.1%)	54 (25.8%)	42 (27.1%)
≥6000	290 (25.2%)	102 (27.7%)	83 (31.4%)	70 (33.5%)	58 (37.4%)
Not provided	76 (6.6%)	22 (6.0%)	15 (5.7%)	11 (5.3%)	8 (5.2%)
		$\chi 2(4) = 1.45$, $p = .84$ $V = 0.03$	$\chi 2(4) = 6.48$ $p = .17$ $V = 0.08$	**$\chi 2(4) = 10.9$, $p = .03$ $V = 0.11$**	**$\chi 2(4) = 17.9$, $p = .001$ $V = 0.17$**

2.2. Measures

All measures were completed by caregivers at the mentioned data collection time-points. No data were retrospectively collected.

2.2.1. Autistic-Like Traits

The *Quantitative Checklist for Autism in Toddlers (Q-CHAT; Allison et al., 2008)* is a 25-item caregiver-report measure of ALTs in toddlers aged 18 to 24 months. It was designed with the purpose of improving on the sensitivity and accuracy of an earlier screening tool for ASD—the *Checklist for Autism in Toddlers* (CHAT; Baron-Cohen et al., 1992). While the original CHAT employed a "Yes (behaviour present) / No (behaviour absent)" binary scoring system, Q-CHAT items are scored dimensionally on a 5-point Likert scale, with scores ranging from 0 to 4. Each numerical value denotes the frequency of the observed behaviour on a continuum. Thirteen of the 25 items are reverse-scored. Minimum and maximum scores obtainable are 0 and 100 respectively, with higher scores indicating higher levels of (i.e. more severe) ALTs. Examples of items include: *"Does your child look at you when you call his/her name?"*, *"Does your child point to share interest with you (e.g. pointing at an interesting sight)?"*, and *"Does your child twiddle objects repetitively (e.g. pieces of string)?"* (see Appendix A for all items of the Q-CHAT, which is freely available online). In the present study, approximately 80-90% of the caregivers who completed the Q-CHAT at the 18-month time-point were the mothers[2].

[2] Respondent data for the Q-CHAT was not obtained at the 18-month time-point. However, 91% of the caregivers who completed the Q-CHAT at 24 months were mothers. Based on this, it is reasonable to estimate that a similar percentage of caregivers who completed the 18-

Preliminary research on the Q-CHAT suggests that it possesses reasonable psychometric properties. In Allison and colleagues' (2008) original study, Q-CHAT total scores obtained from an unselected sample of 754 toddlers in the UK was found to be normally distributed, to have adequate internal consistency (Cronbach's alpha: $\alpha = 0.67$), and good test-retest reliability after one month (intraclass correlation coefficient: ICC = 0.82). Expected between-group differences were also observed: boys obtained significantly higher Q-CHAT scores than girls, and participants diagnosed with ASD obtained significantly higher Q-CHAT scores than participants without ASD (Allison et al., 2008). Other studies which have used the Q-CHAT as a measure of ALTs have also reported similar findings regarding its psychometric properties, gender and case-control differences, and distribution of ALTs in their respective samples (Auyeung, Taylor, Hackett, & Baron-Cohen, 2010; Wong, Huertas-Ceballos, Cowan, & Modi, 2014). At present, there is no published data on the use of the Q-CHAT in Asian populations.

Recently, Magiati and colleagues (in preparation) examined the factor structure of the Q-CHAT in 18-month old Singaporean toddlers from the GUSTO study (most of whom were the same participants as those in the present study) and proposed a three-factor structure of the Q-CHAT: a social-communication (social) traits factor (10 items; score range: 2—27), a non-social/behavioural (non-social) traits factor (8 items; score range: 0—31), and a speech/language factor (4 items; score range: 3—16). This factor structure is consistent with a factor analysis of the Q-CHAT by its original authors

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month Q-CHAT were mothers. This percentage is also consistent with respondent data from other GUSTO measures, where mothers are typically the main respondents.

(Allison et al., 2010). Importantly, the first two factors are congruent with the DSM-5's revised dyadic organization of the diagnostic criteria for ASD (i.e. impairments in social-communication/interaction, and RRBIs). This clustering of social-communication and non-social/behavioural traits is also supported by findings from factor analytic studies of other measures of autistic symptoms in clinical samples (Gotham, Risi, Pickles & Lord, 2007; Matson, Boisjoli, Hess, & Wilkins, 2009; Shuster, Perry, Bebko, & Toplak, 2014).

In this study, social and non-social Q-CHAT factor scores were calculated by adding up the scores of the constituent items of each factor. These factor scores were employed in the main analyses to examine the contributions of the proposed infant predictors in explaining social and non-social ALTs at 18 months. The speech/language factor was not further explored, as it consisted of only four items requesting information about generic, rather than ALT-specific, delays in speech and language development (i.e. *"How many words can your child say?"*; Magiati et al., in preparation). Furthermore, speech/language delays are no longer required for a diagnosis of ASD (American Psychiatric Association, 2013).

2.2.2. Pregnancy and Birth-Related Information

Pregnancy and birth-related information were collected prospectively using two standardized self-report inventories. Participants were asked to complete the first inventory during one of their clinic follow-up appointments at approximately the 26th week of pregnancy. This inventory contained questions about health and life events/habits that may influence pregnancy during the prenatal period, such as whether or not participants consumed

alcohol or smoked during pregnancy. The second inventory was provided before participants were discharged from hospital following their child's successful delivery. The items in this inventory focused on information pertaining to labour, such as whether a C-section was performed, and information about the newborn child such as gender, birth weight, duration of gestation, and birth order in the family. Information from these inventories was documented in the participants' medical records.

As some of the PPOs of interest in this study have been found to co-occur frequently with each other, a composite suboptimality score for the seven PPO risk factors of interest—namely: maternal age ($\geq$ 35 years), gestational age (< 37 weeks), birth weight (< 2500g), birth order (being firstborn vs. later-born), Caesarean delivery (yes), prenatal smoking (yes), and prenatal alcohol consumption (yes)—was calculated to assess overall suboptimality, instead of investigating each factor separately. Computation of this score was performed in a way similar to previous studies (e.g. Bolton et al., 1997; Stein et al., 2006), whereby the absence and presence of each risk factor were assigned scores of '0' and '1' respectively. A single cumulative score ranging from 0 to 7 was obtained, with higher scores indicating higher degrees of birth and obstetric suboptimality (i.e. more PPOs present).

2.2.3. Infant Temperament

The *Carey's Temperament Scales* (CTS; Carey & McDevitt, 1995) are a series of five caregiver-report questionnaires designed to measure temperament in children from 1 month to 12 years of age. The CTS are based on Thomas and Chess' (1977) nine-category model of temperament (see

Introduction, section 1.3.1), with each questionnaire designed to measure temperament-related characteristics over a specific age range. Items consist of statements about the child's behaviour, and caregivers are asked to rate the frequency of the behaviour(s) described in each statement by assigning a numerical score from 1 (almost never) to 6 (almost always). Instead of yielding a single overall composite score, summary scores (range 1 to 6) for each of the nine categories are derived by obtaining the mean score of all answered items for each category (McDevitt & Carey, 1996). Lower scores generally indicate "easier" temperament (less active, more rhythmic, more approaching, more adaptable, reacts more mildly, more positive mood, more persistent, more distractible, and higher response threshold/non-reactive). Conversely, higher scores are largely indicative of more "difficult" temperament (more active, arrhythmic, more withdrawn, slower to adapt, reacts more intensely, more negative mood, less persistent, less distractible, and lower response threshold/more sensitive).

The *Early Infant Temperament Questionnaire* (EITQ; Medoff-Cooper, Carey, & McDevitt, 1993) was used in this study to assess temperament at 3 months of age. It was designed to measure the nine temperament characteristics identified in the NYLS (activity, rhythmicity, approach, adaptability, intensity, mood, persistence, distractibility, and threshold of responsiveness) in 1 to 4-month-old infants. It consists of a total of 76 items and takes approximately 15-20 minutes to complete (see Table 3 for sample items for each dimension on the EITQ and the interpretation of higher/lower scores on each dimension). Most of the items on the EITQ were derived from the *Revised Infant Temperament Questionnaire* (RITQ; Carey & McDevitt,

1978) and modified so as to better reflect the developmental characteristics and abilities of very young infants.

Internal consistencies range from 0.42—0.76 and test-retest reliability coefficients range from 0.43—0.87 across all temperament categories (Medoff-Cooper, Carey, & McDevitt, 1993). The original authors attributed the low reliability coefficients to the inherent challenges of measuring behaviour reliably in very young infants: infants' behavioural styles change as they mature and interact with the environment, and parent ratings of infant temperament naturally become more consistent as they gain more opportunities to observe the infant's behaviour in a greater variety of situations (Medoff-Cooper, Carey, & McDevitt, 1993). At present, no study has evaluated the suitability and/or psychometric properties of the EITQ as a measure of temperament in very young infants in Asia.

Table 3.

Sample items and score interpretation of the EITQ

Dimension	Sample Item	Lower Score	Higher Score
Activity	The infant lies still (little squirming) during hair brushing.*	Inactive	Active
Rhythmicity	The infant's time of waking in the morning varies greatly (by 1 hour or more) from day to day.	Rhythmic	Arrhythmic
Approach	The infant objects (cries, frets) if someone other than main caregiver gives care.	Approaching	Withdrawing
Adaptability	The infant accepts his/her bath any time of day without resisting.*	Adaptable	Non-adaptable
Intensity	The infant's hungry cry is a scream rather than a whimper.	Mild	Intense
Mood	The infant cries during a bowel movement.	Positive	Negative
Persistence	The infant will continuously look at mobile or toy in crib for 5 minutes or more.*	Persistent	Non-persistent
Distractibility	The infant continues to cry when frightened despite several minutes of soothing (picked up, patted).	Distractible	Non-distractible
Threshold	The infant acts the same when the diaper is wet or dry.*	High (i.e. non-reactive)	Low (i.e. sensitive)

* Reverse-scored

2.2.4. Infant Social Development

Gestures. Adapted from the original *MacArthur Communicative Development Inventories* (CDI; Fenson et al., 1994), the *Singapore Early Communicative Development Inventories* (*SECDI*; Tan, 2009) was used in the present study to assess verbal and non-verbal communication in Singaporean infants at 12 months for the variety of English used in Singapore (Low & Brown, 2005). The SECDI is a standardized caregiver-report measure of vocabulary development in children aged 8 to 30 months. It consists of two versions—the *Words and Gestures* (for children aged 8 to 16 months) and the *Words and Sentences* inventories (for infants aged 16 to 30 months).

The *First Communicative Gestures* subsection of the *Words and Gestures* version of the SECDI (SECDI-Words and Gestures) was used in this study to assess gesture use at 12 months. This subsection consisted of 12 items, with each item containing a description of a specific gesture (i.e. "*Requests something by extending arm and opening and closing hand*"), and parents were asked to rate the frequency with which they have observed their child utilizing the gesture described on a three-point frequency scale: "*Not Yet*", "*Sometimes*" and "*Often*". "*Not Yet*" responses were scored 0 while "*Sometimes*" and "*Often*" responses were scored 1 (score range: 0-12).

Mean test-retest reliability of all Gestures scales (including the First Communicative Gestures scale used in this study) of the original CDI was reported to be high at a 1.35-month interval (ICC= 0.86). Although the original researchers reported little formal investigation of the validity of the Gestures scales in infants, they found that scores obtained on these scales were positively associated with performance on communication-related

observational tasks—such as object recognition during play, and understanding of gestures displayed by others—demonstrating some evidence of concurrent validity (Fenson et al., 1994). Currently, there is no local data on the psychometric properties of the *Gestures* subsection of the SECDI, owing to challenges in participant recruitment in Singapore (Tan, 2010).

Imitation/Play and Empathy. The *Imitation/Play* and *Empathy* subscales of the *Infant Toddler Social and Emotional Assessment* (*ITSEA*; Carter & Briggs-Gowan, 2006) were used to assess the development of imitation/play-related skills and empathy at 12 months of age. The ITSEA is a caregiver-report questionnaire designed to measure social-emotional problems and competencies in very young children aged 12 to 36 months. It consists of 139 items distributed across four behavioural domains—*externalizing, internalizing, dysregulation,* and *competencies*—and the whole measure takes approximately 20—30 minutes to complete. Items are rated on a 3-point scale ranging from 0 (*not true/rarely) to 2 (very true/often)*. A *No Opportunity* option is available if parents feel that they have not had the opportunity to observe the behaviour(s) described.

The *Imitation/Play* and *Empathy* subscales used in the present study are part of the *Competence* domain of the ITSEA—which assesses age-appropriate social skills—and consist of 6 and 7 items respectively. Examples of items include *"imitates clapping or waving bye-bye"* for the *Imitation/Play* subscale, and *"tries to make you feel better when you're upset"* for the *Empathy* subscale. Higher scores indicate more adaptive behaviour, and composite subscale scores are derived by obtaining the mean score of all items in that subscale.

An evaluation of the psychometric properties of the ITSEA on children aged 12 to 36 months (N = 214) found the internal consistency of the *Imitation/Play* subscale to be suboptimal (α = 0.59), but that of the *Empathy* subscale to be high (α = 0.82) (Carter, Briggs-Gowan, Jones, & Little, 2003). Despite low reliability values on some of the ITSEA's subscales, strong evidence exists in support of its validity. The ITSEA Competence domain scores were found to be significantly correlated with scores on other well-established measures of infant social and communication development. Competence domain scores correlated significantly with the Socialization (r = .48) and Communication domains (r = .56) of the Vineland Adaptive Behaviour Scales (VABS; Sparrow, Balla, & Cicchetti, 1984), and the Expressive Language (r = .41) and Composite standard scores (r = .47) on the Mullen Scales of Early Learning (MSEL; Mullen, 1995), all with moderate to large effect sizes (Carter et al., 2003). As with the EITQ, no published study has validated the factor structure and psychometric properties of the ITSEA in an Asian infant population.

2.2.5. Socio-demographic information

Participants were requested to provide information about their socio-demographic background, including their ages, educational qualifications, ethnicities, occupations, child gender, and average monthly household income.

2.3. Procedure

Ethical approval for the larger GUSTO study (see Appendix B), within which this study is embedded, was awarded by SingHealth Centralized

Institutional Review Board (CIRB) and National Healthcare Group Domain Specific Review Board (DSRB), and accepted by the National University of Singapore's Institutional Review Board (IRB).

Informed written consent was obtained from eligible participants during the first trimester of pregnancy. Participant eligibility information was obtained from hospital medical records. The nature of the study, including its benefits and risks, was thoroughly explained to eligible candidates prior to consent taking by trained GUSTO research assistants. All participants were informed that they had the right to withdraw from the study at any time. Potential participants were also assured that the standard of care provided to them and their child would not be compromised in any way should they refuse to participate, or decide to withdraw from the study in the future.

Initial follow-ups during pregnancy were conducted in the hospital, with infants being seen at birth, 24 hours after birth, and then at regular intervals up till the child was 2 months of age by trained student interns and research assistants involved in the study at the earlier time-points. Subsequent follow-ups when the children were 3, 6, 9, 12, 15, 24, and 36 months of age took place either at the child's home or at GUSTO's Neurodevelopment Centre—located at Saint Andrew's Community Hospital (SACH). At each follow-up, a variety of observational, experimental, and caregiver self-report measures were administered by research staff. To optimize the data collection process, some of the self-report measures were posted to parents a few weeks before the next follow-up so that they had more time to complete them. The EITQ and Q-CHAT were posted to parents approximately two weeks before their child reached 3 and 18 months of age respectively. Parents were

encouraged to complete the questionnaires and either mail them back to the research team using a return envelope, or hand them in personally during their next scheduled research visit. Parents completed the SECDI and ITSEA during the child's 12-month follow-up visit at the clinic. All participants were financially reimbursed for their participation ($100 per clinic/home visit) and completion of questionnaires ($20 for completing all questionnaires for each time-point).

All GUSTO data were entered into the GUSTO study database by a team of trained research assistants, undergraduate and post-graduate students, including the author, who took the lead in entering, checking and cleaning the 18-month Q-CHAT data. The author was also directly involved in data collection and data entry of other GUSTO Neurodevelopment domain measures at the 18-, 24-, and 36-month time-points as a member of GUSTO's larger research team. As GUSTO is a longitudinal study, some data used in the present thesis—in particular, the early predictors—were collected prior to the commencement of the author's candidature. However, the author contributed to data entry, checking, and cleaning of these variables, in addition to direct data collection of the Q-CHAT and other relevant neurocognitive measures at the aforementioned time-points.

2.4. Statistical analyses

2.4.1. Missing data

Similar to the methods employed by Allison and colleagues (2008), Q-CHAT items that were unanswered or ambiguously scored were conservatively scored '0', and questionnaires with seven or more unanswered

items were excluded from the analyses (n = 1). The EITQ was calculated using computerized scoring software. As the software considered questionnaires to be unrepresentative if 20% or more of the total number of items were missing a response (Carey, 2007), participants who did not answer 15 or more out of the total of 76 items in the EITQ were excluded from the later analyses (n = 3). Missing items on the SECDI were conservatively scored '0'. The original CDI manual did not contain information advising on how missing data should be handled. In the present study, questionnaires with more than one missing item, out of a total of 12, were excluded from the analyses (n = 23) in order to minimize inaccuracies due to missing information.

2.4.2. Preliminary Analyses

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS), Version 21.0. Descriptive statistics were calculated and the data were inspected for normality and outliers. One sample *t*-tests amd chi-square goodness-of-fit tests were used to assess whether there were any differences in scores obtained across all measures, between participants who completed the 18-month Q-CHAT and the full GUSTO sample. Normality of all continuous variables was examined using statistical tests of normality (Shapiro-Wilks' test, as well as skewness and kurtosis coefficients) and inspection of frequency histograms. Internal consistencies of all measures were calculated using Cronbach's alpha.

Relationships between demographic variables and all key study variables were examined using correlational analyses (maternal education and

gender) and one-way ANOVAs (ethnicity) to identify whether any of the demographic variables should be controlled for in the main analyses.

Pearson's correlations were used to examine the relationships between all hypothesized predictor variables to investigate the possibility of multicollinearity between the variables examined in this study, particularly between the temperament dimensions and between the social development variables. Effect sizes were also used for interpreting the strength of findings. Small, moderate and large effect sizes were denoted by correlation coefficients of 0.1, 0.3, and 0.5 respectively (Cohen, 1988). According to Field (2009; pp. 224), correlations exceeding .80 are a cause for concern regarding multicollinearity.

A significance level of 1% was selected as the cut-off for the correlational analyses to adjust for multiple comparisons. Correlation coefficients were not adjusted using the Bonferroni correction as the latter is a very conservative statistical test (Field, 2009). Since this study investigates associations between hypothesized early precursors and trait-related outcomes (as opposed to clinically significant symptoms), only modest to moderate relationship(s) are expected to be observed between the hypothesized predictors and later ALTs, if any at all. Using a slightly less stringent adjustment would therefore achieve greater balance between the risks of committing Type I and Type II errors.

2.4.3. Main Analyses

The first aim of the present study was to investigate whether pregnancy/birth-related complications, temperament at 3 months, and social

development at 12 months were significantly associated with and predicted social and non-social ALTs at 18 months. Pearson correlations were used to examine the associations between these early infancy variables and Q-CHAT social and non-social factor scores.

To maximize utilization of all the data collected from each of the study subsamples, hierarchical multiple regression analyses were conducted to investigate the contribution of each group of predictors (PPOs, infant temperament, infant social development) in explaining Q-CHAT factor scores at 18 months. Demographic variables identified as covariates with Q-CHAT factor scores were entered in Step 1, while the predictor variables of interest were entered in Step 2.

The second aim of this study was to identify unique individual predictors of social and non-social ALTs. To investigate this, one hierarchical regression analysis was each conducted for Q-CHAT social and non-social factor scores. Demographic variables identified as covariates with respective Q-CHAT factor scores were entered in Step 1, while all early infancy variables were entered simultaneously in Step 2.

A significance level of 5% was used in the regression analyses to assess whether variable(s) entered simultaneously in the same step collectively predicted a significant amount of variance in Q-CHAT factor scores, as well as to identify individual variables that uniquely predicted Q-CHAT factor scores.

CHAPTER 3

RESULTS

3.1. Preliminary Analyses

3.1.1. Scale reliabilities, descriptive statistics, and normality

Descriptive statistics of all variables and internal consistencies of the measures employed in this study are presented in Table 4. Internal consistency for total Q-CHAT score was suboptimal (α = 0.53). However, the two Q-CHAT factor scores showed acceptable internal consistencies (social: α = 0.76, non-social: α = 0.69; see Table 4). Internal consistencies for the nine categories of the EITQ ranged from suboptimal to marginally acceptable (α = 0.42—0.66). Good internal consistency was found for the Gestures subsection of the SECDI (α = 0.74). Finally, internal consistencies of the ITSEA were suboptimal for the Imitation/Play subscale (α = 0.54), but acceptable for the Empathy subscale (α = 0.78).

Descriptive statistics were expressed as means and standard deviations for continuous variables, and as numbers and percentages for categorical variables. There were no significant differences in descriptive statistics obtained between participants who completed the Q-CHAT and those in the larger GUSTO study sample, across all variables of interest. Notably, only a very small percentage of mothers reported consuming alcohol (2.9%) or smoking (1.7%) during pregnancy in this sample (see Table 4). However, the possible influences of these variables on later ALTs could still be explored collectively with other PPO risk factors through the composite overall suboptimality score.

Appendix C summarizes the normality statistics of all continuous variables. Shapiro-Wilks' tests of normality were significant for most study variables, suggesting non-normality. However, this statistic generally did not corroborate with other indicators of normality. All variables with significant Shapiro-Wilks' test statistics did not yield standardized skewness or kurtosis coefficients that strongly indicated non-normality. Kim (2013) recommended an absolute critical z-value of 3.29 for making judgments of non-normality in medium-sized samples (N = 50—300), and unstandardized absolute skewness and kurtosis values larger than 2 and 7, respectively, for determining non-normality in large samples (N > 300). Skewness and kurtosis values for all variables, including Q-CHAT scores, did not exceed these aforementioned cut-offs. Moreover, inspection of the frequency histograms of Q-CHAT factor scores revealed an approximately normal distribution, suggesting that deviations from normality, even if present, were small. Thus, it was concluded that the Q-CHAT variables were approximately normally distributed.

Table 4.

Descriptive statistics of study variables and internal consistencies of measures employed

		Participants who completed 18M Q-CHAT		All GUSTO Participants		One-sample *t*-test (*p*) or chi-square (*p*)	Cohen's *d or Kramer's V*
	α	**Mean (SD) or N (%)**	**Range**	**Mean (SD) or N (%)**	**Range**		
PPO complications		**(N = 368)**		**(N = 1089)**			
Maternal age (yrs)	--	30.3 (5.2)	19-44	30.4 (5.2)	18-46	-.27 (.79)	-0.02
Birth Order	--	1.95 (1.0)	1-5	1.87 (.96)	1-8	1.43 (.16)	0.08
Gestational Age (wks)	--	38.3 (1.3)	30-41	38.3 (1.5)	25-41	1.20 (.24)	0.06
Birth Weight (g)	--	3120 (440)	1578-4505	3080 (460)	780-5430	1.52 (.13)	0.08
Prenatal Alcohol (yes)	--	10 (2.7%)	--	20 (1.8%)	--	$\chi 2$ = 1.66 (.20)	--
Prenatal Smoking (yes)	--	6 (1.6%)	--	29 (2.4%)	--	$\chi 2$ = 1.46 (.23)	--
Caesarean Delivery (yes)	--	103 (28%)	--	323 (29.7%)	--	$\chi 2$ = .51 (.48)	--
Overall Optimality	--	1.11 (.98)	0-5	1.15 (1.0)	0-5	-.84 (.40)	-0.04
Temperament (3M)		**(N = 264)**		**(N = 645)**			
Activity	0.42	3.80 (.63)	2-6	3.78 (.64)	2-6	.49 (.62)	0.03
Rhythmicity	0.60	3.31 (.67)	1.4-4.8	3.36 (.63)	1.4-5.1	-1.24 (.22)	-0.08
Approach	0.60	2.77 (.83)	1-5.5	2.82 (.81)	1-5.5	-1.09 (.28)	-0.07
Adaptability	0.53	2.65 (.57)	1.2-4.2	2.68 (.62)	1-4.5	-.94 (.35)	-0.06
Intensity	0.50	3.76 (.86)	1-6	3.75 (.81)	1-6	.29 (.77)	0.02

		Participants who completed 18M Q-CHAT		All GUSTO Participants		One-sample *t*-test (*p*) or chi-square (*p*)	Cohen's *d or Kramer's V*
	α	**Mean (SD) or N (%)**	**Range**	**Mean (SD) or N (%)**	**Range**		
Mood	0.58	2.89 (.64)	1-4.82	2.89 (.62)	1-4.82	.06 (.95)	0.00
Persistence	0.66	2.43 (.71)	1-5.38	2.46 (.70)	1-5.38	-.67 (.50)	-0.04
Distractibility	0.55	2.54 (.73)	1-4.83	2.56 (.72)	1-4.83	-.41 (.68)	-0.03
Threshold	0.64	4.18 (.68)	1.50-6	4.19 (.68)	1.5-6	-.05 (.96)	0.00
SECDI (12M)		**(N = 209)**		**(N = 478)**			
Gestures	0.74	7.71 (2.5)	0-12	7.51 (2.6)	0-12	1.16 (.25)	0.08
ITSEA (12M)		**(N = 209)**		**(N = 493)**			
Imitation/Play	0.54	1.01 (.38)	0-2	1.03 (.38)	0-2	-.70 (.49)	-0.05
Empathy	0.78	.58 (.41)	0-2	.61 (.43)	0-2	-.49 (.62)	-0.03
Q-CHAT (18M)		**(N = 368)**					
Total score	0.53	35.6 (7.2)	12-59	--	--	--	--
Social factor	0.76	9.94 (4.2)	2-27	--	--	--	--
Non-social factor	0.69	13.1 (5.1)	0-31	--	--	--	--

3.1.2. Relationships between demographic variables and the study's main variables

Table 5 summarizes the relationships between demographic variables (child gender, maternal education, and child ethnicity) with (i) the hypothesized predictors and (ii) Q-CHAT factor scores. Pearson's correlations were computed to examine associations with child gender and maternal education, whereas one-way ANOVAs were performed to identify differences in scores obtained on the various measures between ethnic groups. Household monthly income was not included in the subsequent analyses, as it was positively correlated with maternal education with a large effect size (ρ =.62, p <.001; not shown in Table). Hence, only maternal education was used as a proxy measure of participants' socioeconomic standing in this study.

Effect sizes of all statistically significant correlations between demographic variables and key study variables (absolute r = .15—.20) were small. There was also a significant effect of child ethnicity on distractibility at 3 months with a small effect size, $F(2, 260) = 3.66$, $p = .027$, $d = 0.21$. Post-hoc Bonferroni comparisons revealed that found that Indian children (M = 2.75, SD = .83) obtained higher distractibility subscale scores than Chinese children (M = 2.43, SD = .68), indicating that Indian children were significantly less distractible than the Chinese children. However, this effect was only marginally significant (p = .079). In general, most of the hypothesized predictor variables were not significantly correlated with gender or maternal education, and did not differ significantly across ethnicities.

Q-CHAT social factor scores were significantly associated with gender with a small effect size (r = -.15; see Table 5). Female infants were reported

by their caregivers to have fewer (less severe) social-communication ALTs than male children. There was a significant effect of child ethnicity on Q-CHAT social factor scores with a small effect size, $F(2, 364) = 3.51$, $p = .03$, $d = 0.20$. Post-hoc Bonferroni comparisons showed that Chinese infants ($M = 10.4$, $SD = 4.4$) had somewhat higher social-communication related ALTs than Indian infants ($M = 8.89$, $SD = 3.7$), but this difference only just reached statistical significance ($p = .049$). Q-CHAT non-social factor scores were inversely associated with maternal education with a small effect size ($r = -.16$, see Table 5), indicating that less educated mothers tended to report somewhat higher levels of non-social ALTs in their children.

Overall, these findings indicate that the key study variables were generally not significantly influenced by socio-demographic variables. Effect sizes of all significant effects/relationships associated with demographic differences were small.

Table 5.

Relationships between demographic variables and key study variables

	Child Gender[a] (Pearson's *r*)	Maternal Education[b] (Pearson's *r*)	Child Ethnicity[c] *F*(*sig*)
Pregnancy/birth-related factors			
Total suboptimality	**.15****	.12*	2.30 (.10)
Temperament (3 months)			
Activity	-.00	-.03	0.04 (.96)
Rhythmicity	.09	**-.20****	2.37 (.10)
Approach	-.11	-.00	1.46 (.23)
Adaptability	-.03	**-.16****	0.22 (.80)
Intensity	.04	.05	3.02 (.50)
Mood	-.07	.01	0.27 (.76)
Persistence	.02	-.09	2.19 (.11)
Distraction	-.04	-.11	**3.66 (.03)**
Threshold	-.01	.09	1.42 (.24)
SECDI-Words and Gestures (12 months)			
12M Gestures total score	.12	-.13*	1.15 (.32)
ITSEA Competence Subscales (12 months)			
12M Imitation/Play	-.08	.15*	0.11 (.89)
12M Empathy	-.00	.06	1.11 (.33)
Q-CHAT factor scores (18 months)			
Social	**-.15****	.01	**3.51 (.03)**
Non-social	.01	**-.16****	2.05 (.13)

[a] Gender was coded as 1 = male and 2 = female

[b] Maternal education was coded as 1 = none/primary, 2 = secondary/ITE/NTC, 3 = pre-university/diploma, 4 = university

[c] Ethnicity was coded as 1 = Chinese, 2 = Malay, 3 = Indian

* $p < .05$ (2-tailed), ** $p < .01$ (2-tailed), in bold; no correlations were $p < .001$.

3.1.3. Intercorrelations between the hypothesized infancy predictors

Table 6 summarizes the bivariate correlations among all hypothesized predictors. Several significant associations, with small to large effect sizes, (range of absolute *r*s = .17—.54) were observed between the nine temperament categories of the EITQ. The ITSEA *Imitation/Play* and *Empathy* subscales were positively correlated with each other with a large effect size (*r* = .55). Despite the presence of statistically significant associations between the constituent scales of both the EITQ and ITSEA, the magnitudes of these correlations (all *r*s <.80) were not sufficiently large to suggest that any two subscales within either of these measures might be measuring the same underlying construct (Field, 2009). Hence, the subscales of the EITQ and ITSEA were considered distinct, but related, constructs.

Two significant correlations were observed between variables belonging to different categories of early precursors. Total suboptimality was positively associated with mood at 3 months with a small effect size (*r* = .16), indicating that mothers who had a greater number of birth/obstetric complications tended to rate their infants as having more negative mood at 3 months. Also, infants' mood at 3 months was negatively associated with gestures at 12 months with a small effect size (*r* = -.19), showing that infants who were reported to have more positive mood at 3 months used more gestures at 12 months. There were no other statistically significant relationships between suboptimality, temperament at 3 months, and gestures, imitation/play, and empathy at 12 months of age.

Table 6.

Intercorrelations among the hypothesized infancy predictor variables

	SUB-OP	ACT	RHY	APP	ADP	INT	MOOD	PERS	DIST	THRE	GEST	IM/P	EMP
SUB-OP		.07	.04	.02	.01	.13*	**.16****	.05	.01	-.09	-.08	.03	.01
ACT			.12*	.04	**.23****	**.27****	**.20****	.01	.15*	.08	-.04	.05	.01
RHY				-.05	**.21****	.03	.11	**.19****	**.23****	-.09	-.04	.01	.12
APP					**.28****	**.19****	**.25****	.13*	**.26****	**.17****	.09	-.04	.01
ADP						.13*	**.50****	**.33****	**.54****	-.12	.03	.06	.02
INT							**.29****	**-.20****	**.25****	**.32****	-.01	.01	-.00
MOOD								**.39****	**.50****	-.12*	**-.19****	.07	.04
PERS									**.35****	**-.41****	-.13	.17*	.05
DIST										-.15*	.00	.07	-.01
THRE											.11	-.06	-.04
GEST												-.08	-.04
IM/P													**.55****
EMP													

Key: SUB-OP=Overall suboptimality; ACT=activity; RHY=rhythmicity; APP=approach; ADP=adaptability; INT=intensity; MOOD=mood; PERS=persistence; DIST=distractibility; THRE=threshold; GEST=gestures; IM/P=imitation/play; EMP=empathy.

* $p < .05$ (2-tailed); **** $p < .01$ level (2-tailed) in bold; no correlations were $p < .001$.**

3.1.4. Summary of findings from the preliminary analyses

The preliminary analyses identified a number of findings impacting the main analyses to follow. Firstly, the subsamples utilized in this study were generally demographically representative of the full GUSTO sample. This suggests that the findings from the main analyses are likely to be generalizable to the larger GUSTO sample, as well as to the general population.

Secondly, although the internal consistencies of several subscales of the EITQ and ITSEA had suboptimal ($\alpha < 0.65$), the alpha coefficients obtained in this study were, in fact, similar to the range of values reported in validation studies of these measures by the original researchers (Medoff-Cooper et al., 1993; Carter et al., 2003). Internal consistencies of Q-CHAT factor scores were found to be within acceptable ranges.

The Q-CHAT total score was not used as one of the outcome measures in this study for two reasons. The first was that its internal consistency was found to be suboptimal ($\alpha = 0.53$) and lower than that obtained in Allison and colleagues' (2008) original study ($\alpha = 0.67$). The second was because the present study aimed to examine early predictors separately for social and non-social ALTs. Hence, Q-CHAT total scores were not analyzed further. Inter-correlations between the key study variables revealed no evidence of multicollinearity (i.e. $r \geq .80$).

Finally, the analyses identified gender as a covariate of Q-CHAT social factor scores and maternal education as a covariate of Q-CHAT non-social factor scores in the study sample. Hence, these demographic variables were subsequently controlled for in the main analyses.

3.2. Main analyses

Table 7 provides a summary of the correlations between the hypothesized predictors and Q-CHAT social and non-social factor scores.

3.2.1. Associations between hypothesized predictors and Q-CHAT factor scores

Negative mood (r = .19), lower distractibility (r = .18) and lower persistence (r = .24) at 3 months were associated with higher Q-CHAT social factor scores (i.e. more social ALTs) with small effect sizes. Higher threshold of responsiveness (r = -.21) at 3 months and lower gesture scores at 12 months (r = -.29) were associated with higher Q-CHAT social factor scores, all with small effect sizes. Higher activity and lower distractibility (both r = .21) were associated with higher Q-CHAT non-social factor scores at 12 months with small effect sizes (see Table 7).

A number of the hypothesized predictors were not significantly associated with Q-CHAT factor scores at 18 months. Overall suboptimality Imitation/Play, and Empathy scores were not correlated with Q-CHAT factor scores. Moreover effect sizes of these non-significant associations were small (all absolute rs < .12; all ps > .05). However, given the considerable empirical evidence from high-risk sibling studies that has implicated these aspects of early social competence with autistic symptoms, the latter group of predictors was still included in the subsequent regression analyses.

Table 7.

Correlations between hypothesized predictors and 18-month Q-CHAT factor scores

	Q-CHAT social factor score	Q-CHAT non-social factor score
Pregnancy/birth-related factors		
Overall suboptimality	.04	.03
Temperament (3M)		
Activity	-.13*	**.21****
Rhythmicity	.12*	.11
Approach	.04	.06
Adaptability	.09	.14*
Intensity	-.02	.11
Mood	**.19****	.08
Persistence	**.24****	.02
Distractibility	**.18****	**.21****
Threshold	**-.21****	-.08
SECDI-Words and Gestures (12M)		
Gestures total score	**-.29****	.08
ITSEA Social Competence (12M)		
Imitation/Play	-.10	.05
Empathy	-.12	.05

*$p < .05$ (2-tailed), ** **$p < .01$ (2-tailed) in bold; no correlations were $p < .001$.**

3.2.2. PPO suboptimality as a single predictor of 18-month Q-CHAT factor scores

Tables 8 and 9 show the hierarchical regression analyses testing for the overall effect of prenatal, perinatal, and obstetric (PPO) suboptimality scores on 18-month Q-CHAT social and non-social factor scores respectively.

Predictors of Q-CHAT social ALTs. Child gender was entered into the model at Step 1, while overall suboptimality score was entered in Step 2. The final model R^2 was significant, but explained only a very small percentage (2.3%) of the variance in Q-CHAT social factor scores: $R^2 = .023$, $F(2, 365) =$

4.21, p = .016. Step 1 was significant, with gender explaining 1.9% of the variance, R^2 = .019, F(1, 366) = 7.10, p = .008. In Step 2, the addition of overall PPO suboptimality did not significantly increase the predictive utility of the model, R^2 = .023, ΔR^2 = .004, F_{change} (1, 365) = .25, p > .05 *(ns)* (see Table 8). Thus, only gender significantly predicted Q-CHAT social factor scores when both these variables were entered together.

Table 8.

Hierarchical regression analyses assessing overall suboptimality as a predictor of 18-month Q-CHAT social factor scores (N=368)

	B	***SE (B)***	***B***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.019*	--
Gender	-1.16	.43	-.14**		
Step 2				.023	.004
Gender	-1.23	.44	-.15**		
Overall suboptimality	.26	.22	.06		
*p < .05; **p < .01; ***p < .001					

Predictors of Q-CHAT non-social ALTs. Maternal education was entered into the model at Step 1, while overall suboptimality score was entered in Step 2. The final model R^2 was non-significant, R^2 = .034, F(2, 365) = 6.35, p > .05 *(ns)*. Step 1 was significant, with maternal education explaining 3.2% of the variance, R^2 = .032, F(1, 366) = 11.9, p = .001. In Step 2, the addition of overall PPO suboptimality did not significantly increase the predictive utility of the model, R^2 = .032, ΔR^2 = .002, F_{change} (1, 365) = .78, p > .05 *(ns)* (see Table 9). Thus, only maternal education significantly predicted Q-CHAT non-social factor scores when both these variables were entered together.

Table 9.

Hierarchical regression analyses assessing overall PPO suboptimality as a predictor of 18-month Q-CHAT non-social factor scores (N=368)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.032	--
Maternal Education	-.91	.26	-.18**		
Step 2				.034	.002
Maternal Education	-.94	.27	-.18***		
Overall suboptimality	.24	.27	.05		
*$p < .05$; **$p < .01$; ***$p < .001$					

3.2.3. Temperament at 3 months as a predictor of 18-month Q-CHAT factor scores

Hierarchical regression analyses evaluating the influence of the nine temperament dimensions on Q-CHAT social and non-social factor scores in 264 participants from the GUSTO study, for whom data on both temperament and ALTs were available, are shown in Tables 10 and 11 respectively. Demographic variables that significantly correlated with Q-CHAT factor scores were entered in Step 1, while all nine temperament dimension variables were entered simultaneously in Step 2.

Predictors of Q-CHAT social ALTs. Child gender was entered into the model in Step 1, while the nine temperament dimension variables were entered as a block in Step 2. The final model R^2 was significant and explained 15.3% of the variance in Q-CHAT social factor scores, $R^2 = .15$, $F(9, 253) = 4.57$, $p < .001$ (see Table 10). Step 1 was significant, $R^2 = .025$, $F(1, 262) = 6.66$, $p = .01$, showing that gender significantly accounted for 2.5% of the variance. Collectively, the temperament dimension variables entered in Step 2

significantly increased the predictive utility of the model, explaining an additional 12.8% of the variance above and beyond that explained by gender alone, $\Delta R^2 = .13$, $F_{change}(9, 253) = 4.25$, $p < .001$. In the final model, gender, activity, and threshold of responsiveness specifically emerged as unique predictors of Q-CHAT social factor scores at 18 months when all temperament predictors were considered together (Table 10).

Table 10.

Hierarchical regression analyses assessing 3-month temperament as a predictor of 18-month Q-CHAT social factor scores (N=264)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\Delta\mathbf{R^2}$
Step 1				.025*	--
Gender	-1.35	.52	-.16*		
				.15***	.13***
Step 2					
Gender	-1.42	.50	-.17**		
Activity	-1.18	.42	-.18**		
Rhythmicity	.67	.39	.11		
Approach	.01	.33	.00		
Adaptability	-.56	.57	-.08		
Intensity	.27	.35	.05		
Mood	.82	.52	.12		
Persistence	.80	.45	.13		
Distractibility	.38	.44	.07		
Threshold	-.88	.43	-.14*		

*$p < .05$; **$p < .01$; ***$p < .001$

Predictors of Q-CHAT non-social ALTs. Maternal education was entered into the model at Step 1, while the nine temperament dimension variables were entered as a block in Step 2. The final model R^2 was significant and explained 12.1% of the variance in Q-CHAT scores, $R^2 = .12$, $F(10, 253) = 3.47$, $p < .001$ (see Table 11). Maternal education (Step 1) significantly accounted for 3.7% of the variance, $R^2 = .037$, $F(1, 262) = 10.0$, $p = .002$. All

temperament variables were entered in Step 2 and significantly increased the predictive utility of the model, explaining an additional 8.4% of the variance above and beyond that explained by maternal education alone, $\Delta R^2 = .084$, $F_{change}(9, 253) = 2.68$, $p = .005$. In the final model, activity and distractibility specifically emerged as significant unique predictors of Q-CHAT non-social factor scores at 18 months when all temperament predictors were considered together (Table 11).

Table 11.

Hierarchical regression analyses assessing 3-month temperament as a predictor of 18-month Q-CHAT non-social factor scores (N=264)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.037**	--
Maternal Education	-.98	.31	-.19**		
Step 2				.12***	.084**
Maternal Education	-.83	.31	-.16**		
Activity	1.42	.51	.18**		
Rhythmicity	.27	.48	.04		
Approach	.20	.40	.03		
Adaptability	-.21	.70	-.02		
Intensity	.36	.43	.06		
Mood	-.46	.63	-.06		
Persistence	-.60	.54	-.08		
Distractibility	1.38	.54	.20*		
Threshold	-.82	.52	-.11		

*$p < .05$; **$p < .01$; ***$p < .001$

3.2.4. Social/communication development at 12 months as a predictor of 18-month Q-CHAT factor scores

Hierarchical regression analyses evaluating the influence of gestures, imitation/play, and empathy at 12 months on Q-CHAT social and non-social factor scores at 18 months for a subsample of 209 participants, for whom data

on both these predictor and outcome variables were available, are shown in Tables 12 and 13 respectively. Demographic variables that significantly correlated with factor scores were entered in Step 1, while all social development variables at 12 months (gestures, imitation/play, and empathy) were entered simultaneously in Step 2.

Predictors of Q-CHAT social ALTs. Child gender was entered into the model at Step 1, while gestures, imitation/play and empathy scores were entered as a block in Step 2. The final model was significant and explained 14.2% of the variance in Q-CHAT social factor scores, $R^2 = .14$, $F(4, 204) = 8.42$, $p < .001$ (Table 12). Gender (Step 1) did not significantly account for variance in Q-CHAT social factor scores, $R^2 = .013$, $F(1, 207) = 2.68$, $p > .05$ *(ns)*. The 12-month social development variables entered in Step 2 significantly increased the predictive utility of the model, explaining an additional 12.9% of the variance, $\Delta R^2 = .13$, $F_{change}(3, 204) = 10.2$, $p < .001$. Only gestures at 12 months emerged as a unique predictor of Q-CHAT social scores when all social development variables were considered together (see Table 12).

Table 12.

Hierarchical regression analyses assessing 12-month social development as a predictor of 18-month Q-CHAT social factor scores (N=209)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.013	--
Gender	-.98	.60	-.11		
Step 2				.14***	.13***
Gender	-.68	.57	-.08		
Gestures	-.57	.12	-.32***		
Imitation/Play	-.35	.90	-.03		
Empathy	-1.60	.82	-.15		
*$p < .05$; **$p < .01$; ***$p < .001$					

Predictors of Q-CHAT non-social ALTs. Maternal education was entered into the model at Step 1, while gestures, imitation/play and empathy scores were entered simultaneously in Step 2. The final model was significant and explained 4.8% of the variance in Q-CHAT non-social factor scores, R^2 = .048, $F(4, 204) = 2.55$, $p = .041$ (Table 13). Maternal education (Step 1) significantly accounted for 4.0% of the variance in Q-CHAT non-social factor scores, $R^2 = .04$, $F(1, 207) = 8.72$, $p = .004$. However, Step 2 showed that gesture, imitation/play and empathy did not account for additional variance beyond that which was already explained by maternal education, $\Delta R^2 = .007$, $F_{change}(3, 204) = .51$, $p = .68$ (*ns*; see Table 13). Thus, the 12-month social development measures did not predict Q-CHAT non-social factor scores at 18 months.

Table 13.

Hierarchical regression analyses assessing 12-month social development as a predictor of 18-month Q-CHAT non-social factor scores (N=209)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.040**	--
Maternal Education	-1.08	.37	-.20**		
Step 2				.048	.007
Maternal Education	-1.12	.38	-.21**		
Gestures	.03	.14	.01		
Imitation/Play	.57	1.12	.04		
Empathy	.67	1.01	.05		
*$p < .05$; **$p < .01$; ***$p < .001$					

3.2.5. Predicting 18-month Q-CHAT factor scores from all predictors in the first year of life

The multiple hierarchical regression analyses evaluating the influence of PPOs, temperament at 3 months, gestures, imitation/play, and empathy at 12 months on Q-CHAT social and non-social factor scores for the 155 children who had all measures complete at all time-points examined in the present study are presented in Tables 14 and 15 respectively. Demographic variables that significantly correlated with Q-CHAT factor scores were entered in Step 1, while all hypothesized predictors were entered as a block in Step 2.

Predictors of Q-CHAT social ALTs. Gender was entered into the model at Step 1, while overall suboptimality, 3-month temperament variables, and 12-month social development variables were entered simultaneously in Step 2. The final model was significant and explained 28.6% of the variance in social factor scores, $R^2 = .29$, $F(14, 140) = 4.02$, $p < .001$ (see Table 14).

Gender (Step 1) did not significantly account for the variance in Q-CHAT social factor scores, R^2 = .02, $F(1, 153) = 2.38$, $p > .05$ *(ns)*. Collectively, this study's hypothesized early predictors (Step 2) significantly accounted for 27.1% of the variance above and beyond that explained by gender, $\Delta R^2 = .27$, $F_{change}(13, 140) = 4.10$, $p < .001$. Activity at 3 months and gestures at 12 months specifically emerged as unique significant predictors of Q-CHAT social factor scores at 18 months, when all early infancy predictors in the first year of life were considered together (Table 14).

Table 14.

Multiple regression analyses assessing overall obstetric suboptimality, temperament at 3 months, and social development at 12 months as predictors of 18-month Q-CHAT social scores (N = 155)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.015	--
Gender	-1.08	.70	-.12		
Step 2				.29***	.27***
Gender	-.77	.65	-.09		
Overall suboptimality	.16	.36	.03		
Activity	-1.22	.54	-.17*		
Rhythmicity	.64	.51	.10		
Approach	.06	.41	.01		
Adaptability	.49	.73	.07		
Intensity	.70	.44	.14		
Mood	.18	.69	.03		
Persistence	.67	.54	.11		
Distractibility	.45	.56	.08		
Threshold	-.12	.58	-.02		
Gestures	-.69	.14	-.38***		
Imitation/Play	-1.00	1.01	-.09		
Empathy	-.84	.99	-.08		

*$p < .05$; **$p < .01$; ***$p < .001$

Predictors of Q-CHAT non-social ALTs. Maternal education was entered into the model at Step 1, while overall suboptimality, 3-month temperament dimensions, and 12-month social development variables were entered simultaneously in Step 2. The final model R^2 was significant and explained 18.1% of the variance in Q-CHAT non-social factor scores, R^2 = .18, $F(14, 140) = 2.20$, $p = .01$ (see Table 15). Maternal education (Step 1) significantly accounted for 4.2% of the variance, $R^2 = .042$, $F(1, 153) = 6.67$, $p = .011$. Collectively, this study's hypothesized early predictors (Step 2) significantly accounted for 13.9% of the variance above and beyond that explained by maternal education, $\Delta R^2 = .14$, $F_{change}(13, 140) = 1.82$, $p = .045$. Maternal education, and adaptability and distractibility at 3 months specifically emerged as unique significant predictors of Q-CHAT non-social factor scores at 18 months, when all early infancy predictors in the first year of life were considered together (Table 15).

Table 15.

Multiple regression analyses assessing overall obstetric suboptimality, temperament at 3 months, and social development at 12 months as predictors of 18-month Q-CHAT non-social factor scores (N = 155)

	B	***SE (B)***	***β***	$\mathbf{R^2}$	$\mathbf{\Delta R^2}$
Step 1				.042*	--
Maternal Education	-1.13	.44	-.20*		
Step 2				.18*	.14*
Maternal Education	-1.17	.46	-.21*		
Overall suboptimality	.06	.46	.01		
Activity	.88	.69	.10		
Rhythmicity	.54	.65	.07		
Approach	.21	.51	.04		
Adaptability	-1.93	.92	-.22*		
Intensity	.31	.56	.05		
Mood	.73	.88	.09		
Persistence	-.49	.70	-.07		
Distractibility	1.35	.72	.20		
Threshold	-1.68	.74	-.20*		
Gestures	.23	.18	.11		
Imitation/Play	.97	1.38	.07		
Empathy	.74	1.24	.06		

*$p < .05$; **$p < .01$; ***$p < .001$

CHAPTER 4

DISCUSSION

The present study examined the relationships between a number of early infancy variables thought to be possible precursors of ASD/ALTs, and the later emergence of social and non-social ALTs in an unselected sample of 368 toddlers. Specifically, it investigated whether these hypothesized precursors, spanning from gestation to 12 months of age, were associated with and predicted social and non-social ALTs at 18 months of age. The main objectives for this thesis were: (i) to explore whether the associations between these early precursors and later autistic traits/symptoms—which have mostly been reported in clinical and high-risk sibling studies—extend also to the broader continuum of ALTs found in the general population, (ii) to test whether social and non-social ALTs at 18 months were associated with and predicted by different early infancy variables, and (iii) to provide a preliminary evaluation of the degree to which the associations between early precursors and later ALTs, which have been based on Western samples, may be generalized to an unselected Asian community sample. It was hypothesized that the variables of interest in each category of early precursors (PPO risk factors, 3-month infant temperament, and 12-month infant social development) would be significantly associated with and predictive of ALTs at 18 months. Additionally, it was hypothesized that the 12-month social precursors would be related to and predictive of only social ALTs. There is currently no evidence to make an informed prediction regarding whether PPOs or temperament may exhibit differential associations with social versus non-

social ALTs. Hence, the separate analyses for these variables on social and non-social ALTs were exploratory.

4.1. Summary of key findings

The present study, embedded within the larger GUSTO study, examined (i) PPO complications, (ii) infant temperament at 3 months, and (iii) gesture use, imitation/play skills, and empathy at 12-months as infancy predictors of later autistic traits at 18 months in an unselected sample of 368 Singaporean toddlers who were followed up longitudinally. The main findings of this study were that early infancy precursors within the first 12 months of life were predictive of ALTs at 18 months and that social and non-social ALTs were associated with and predicted by different precursors.

Specifically, no significant associations were observed between overall obstetric suboptimality scores and social as well as non-social ALTs across all analyses. When 3-month temperament was examined as a predictor alone, lower activity and higher threshold of responsiveness predicted more social ALTs at 18 months, whereas higher activity and distractibility predicted more non-social ALTs. When 12-month infant social precursors were considered alone, only gesture use uniquely predicted social ALTs—specifically, less gesture use predicted more social ALTs. No significant relationships were observed between infant social precursors at 12 months and non-social ALTs at 18 months.

When all infancy predictors were considered together, activity at 3 months and gesture use at 12 months emerged as unique predictors of social

ALTs at 18 months. Adaptability and threshold of responsiveness at 3 months emerged as unique predictors of non-social ALTs at 18 months.

4.2. PPO complications were not significant predictors of later ALTs

Contrary to Hypothesis 1, overall obstetric suboptimality was neither significantly associated with nor predictive of social and non-social ALTs at 18 months in this unselected sample. To date, only one UK-based study has examined the association between PPOs and ALTs in a population-based sample of unselected twin pairs aged 7- to 8-years (Ronald et al., 2010). Ronald and colleagues reported a modest but significant relationship between PPOs and later ALTs at that age. This study did not find a significant relationship between PPOs and later ALTs, as was reported in Ronald and colleagues' (2010) study. Given that the relationships between PPOs and autistic trait variability are expected to be modest, it is possible that the smaller sample size employed in this study relative to Ronald et al. (N = 13690) may have hindered the detection of true associations owing to limited statistical power. Another possible reason might be that the present study only studied seven PPO variables. In comparison, Ronald and colleagues (2010) sampled a much wider range of approximately 30 such variables. The small number of PPO variables investigated in this study may therefore have limited the variability in scores obtained on the measure of overall suboptimality.

In a discussion of their findings, Ronald and colleagues (2010) suggested that perhaps the associations between PPOs and ALTs may have been more salient if the latter were assessed at an earlier time-point. The present study explored this possibility by measuring ALTs at 18 months, much

earlier than Ronald and colleagues (7-8 years), while using a similar method for assessing PPO suboptimality. Contrary to Ronald and colleagues suggestions, the results of this study suggest that the strength of the associations between PPOs and later ALTs may not be contingent on the age at which ALTs are measured/studied.

The lack of significant findings could also be due to the fact that the cut-offs used to determine the presence of risk are based on Western studies, and possibly not appropriate in our culture. For example, although LBW has generally been defined as < 2500g, a US-based study has found that typical Asian neonates are smaller than their Western counterparts (Madan, Holland, Humbert, & Benitz, 2001), highlighting the need to take ethnic differences into consideration when determining the risk thresholds of PPOs.

A further possibility for the present findings, considered in the light of existing literature, is that PPOs may play a significant role in predicting ALTs only at the quantitative extreme end of the autistic severity spectrum, but not at the non-extreme ranges of the continuum. It has been suggested that at least part of the etiological contribution of PPOs on ALTs occurs through interaction with genetic factors (Lundstrom et al 2012; Ronald & Hoekstra, 2010). Moreover, it is clear from studies in clinical samples that PPOs are associated with higher risk of later ASD (for reviews, see: Bilder et al., 2009; Gardener et al., 2009; Gardener et al., 2011; Kolevzon et al., 2007; Sandin et al., 2013). Thus, it is possible that the etiological contributions of PPOs to autistic trait variability may be more salient at high levels of ALTs, which in turn, is more likely to arise in the context of greater genetic vulnerability. As the sample size of the present study was not sufficiently large, it was not

possible to conduct a separate investigation of the association between PPOs and ALTs in the extreme range.

4.3. Different infancy temperament dimensions as early as 3 months were associated with and predicted later social vs. non-social ALTs.

Findings related to infant temperament generally supported both study hypotheses. Collectively, temperament-related variables at three months of age significantly predicted both social and non-social ALTs at 18 months, regardless of whether temperament was considered alone or together with the other study variables. In addition, different temperament dimensions emerged as unique predictors of social and non-social ALTs at 18 months in all relevant analyses. Furthermore, different dimensions of temperament were found to significantly predict social and non-social ALTs at 18 months.

Lower activity at 3 months was found to be associated with more social ALTs at 18 months. This finding is closely similar to, and extends, that of Bolton and colleagues (2012), who also reported that lower activity at 6 months was associated with higher ALTs at 30 months in their sample of approximately 14000 children from England, who were followed up longitudinally from gestation. This finding is also congruous with work by del Rosario and colleagues (2014), who reported that high-risk infant siblings subsequently diagnosed with ASD were less active at 6 and 12 months of age compared to high-risk children who did not develop ASD. Thus, the results of this study extend existing findings by showing that temperament even earlier in life (3 months) may be predictive of later ALTs than previously established. The presence of this association may be related to the items in the Activity

dimension of the EITQ. Although this dimension purports to assess an infant's general level of motor activity, some of its items assess an infant's level of physical activity in situations that involve some form of social or sensory/ physical interaction with another social agent (e.g. dressing or bathing). It is therefore possible that low levels of activity may reflect reduced interest in social interaction.

Conversely, higher activity at 3 months predicted more non-social ALTs in the second year of life. Although this association was not reported in studies using population (Bolton et al., 2012) or high-risk infant samples (del Rosario et al., 2014), it was congruent with the results reported by Brock and colleagues (2012), who found that 3 to 7-year-old children with ASD had significantly higher activity levels than typically developing children. This finding might also be plausibly explained by considering previous literature together with the nature of the items in the Activity subscale. Brock and colleagues (2012) have proposed that high activity may be an indicator of sensory hyperresponsiveness, and some items in the Activity subscale involve situations with sensory stimulation. It could be that infants' heightened level of activity during these situations (such as hair brushing, nail-cutting, and bathing) are indicative of their discomfort with the non-social/environmental stimuli involved in these activities (Baranek et al., 2007).

Higher threshold of responsiveness at 3 months (i.e. lower sensitivity) also predicted more social ALTs in the second year of life. This resonates with the earlier finding that threshold at 6 months predicted ALTs at 30 months in an unselected sample (Bolton et al., 2012). Similar relationships were also observed in high-risk infants later diagnosed with ASD (del Rosario et al.,

2014), and in case-control comparisons of 3 to 8-year-old children with ASD versus healthy controls (Brock et al., 2012; Hepburn & Stone, 2006). However, it runs contrary to observations by parents and teachers that children with ASD tend to react very strongly to minor environmental changes (Rogers, Hepburn, & Wehner, 2003). Hepburn and Stone (2006) suggested that this discrepancy could be because the items in the *Threshold* subscale assess responsiveness to both social (e.g. "*The infant notices (reacts differently) to a change in person giving care"*) and non-social (e.g. *"The infant notices (startles) sudden movements or bumps when in stroller or carriage."*) environmental stimuli (Hepburn & Stone, 2006). Inspection of the 10 items in the Threshold subscale reveal that approximately half of the items involve a social stimulus or social agent. Since children with ASD have been reported to exhibit deficits in responses to social stimuli (Dawson et al., 2004), it is plausible that children with more social ALTs in toddlerhood are relatively less responsive to social stimuli earlier in life. One way in which future investigations seeking to elucidate the association between response threshold and social/non-social ALTs may be refined would be to create separate threshold subscales based on the nature of the stimuli (social vs. non-social) in the item, and to use only the relevant subscale for each autistic trait cluster.

Reduced distractibility at 3 months was predictive of more non-social ALTs in the second year of life. This finding was not obtained in earlier studies in population-based samples (Bolton et al., 2012) or high-risk samples (del Rosario et al., 2014), which found higher distractibility at 6 months to be linked with more ALTs/higher risk of later ASD respectively. However, Bolton and colleagues (2012) found that children in their study who were later

diagnosed with ASD exhibited significantly reduced distractibility at 24 months relative to TD children. Furthermore, reduced distractibility was also reported by Brock and colleagues (2012) in older children (3- to 7-years-old) with ASD, relative to TD children. Brock and colleagues proposed that low distractibility in children with ASD may be associated with a lack of responsiveness to changes in one's ongoing sensory experiences. That infants who were less distractible at 3 months tended to present with more non-social ALTs at 18 months is congruent with previous research—low distractibility has been described as reflecting a lack of responsivity to changes in one's ongoing sensory experiences, a trait commonly implicated in ASD (Hepburn & Stone, 2006).

An unexpected finding was that higher adaptability at 3 months was linked with more non-social ALTs in the second year of life. This contradicts previous findings that have investigated the relationship between adaptability and later ALTs/ASD risk, all of which have found lower adaptability to be predictive of more ALTs (Bolton et al., 2012), or to characterize the temperament of high-risk infants and children diagnosed with ASD (Brock et al., 2012; del Rosario, 2014; Hepburn & Stone, 2006). The items on the Adaptability subscale of the EITQ assess the ease with which the infant copes with changes in daily activities. Given that having strong preferences for sameness and routine are characteristic traits of individuals diagnosed with ASD (Hepburn & Stone, 2006), it was expected that lower, rather than higher, levels of adaptability, would predict later non-social ALTs at 18 months. It is possible that the present study's findings may be due to misinterpretation of the test items, as a result of the close overlaps in item content with other

subscales. For example, specific items on the Adaptability subscale (*"The infant resists (squirms, pulls away) during hair brushing"*) are closely similar to items in the Activity subscale *("The infant lies still (little squirming) during hair brushing"*). A further example can be found between an item on the Adaptability subscale (*"The infant resists changes in feeding schedule (1 hour or more) even after two tries"*) and the Approach subscale (*"The infant accepts right away a change in time of feeding"*). These overlaps in content between items from different subscales in the EITQ may therefore have contributed to less accurate estimates of the adaptability dimension.

Finally, the present study did not find lower persistence at 3 months to be predictive of higher social or non-social ALTs at 18 months. Conversely, earlier studies on population and clinical samples of infants/children, ranging from 30 months to 8 years of age, have found lower persistence to be associated with more ALTs/higher risk of ASD (Bolton et al., 2012; Brock et al., 2012; Hepburn & Stone, 2006). Since this study measured ALTs at 18 months—much earlier compared to other studies—one possible reason could be that the potential influence of the persistence dimension of temperament on ALTs may become more salient slightly later in life. A further possibility could be that the lack of a persistence—ALT association might have been due to differences in the methods employed in this study relative to other studies. Specifically, the effect of persistence may have been attenuated as this study investigated temperament in relation to specific ALT domains rather than overall levels of ALTs. However, further study is necessary before any conclusions can be drawn.

4.4. Early social development at 12 months a predictor of later ALTs: only gestures predicted later social ALTs

4.4.1. Gestures significantly predicted social ALTs only

An important pair of findings in this study, consistent with the study hypotheses, was the presence of a significant inverse association between gesture use at 12 months and social ALTs at 18 months and the absence of an association between gesture use at 12 months and non-social ALTs at 18 months. This finding is aligned with that of Bolton and colleagues' (2012), who reported that gesture use at 15 months was predictive of ALTs at 30 months. The results of this study extend this finding, supporting the predictive utility of gesture use at an earlier time-point. In addition, this finding is congruous with those of studies on children at risk of ASD, which have found poor gesture development to be more common in high-risk infant siblings (Ibañez et al., 2013) and identified it as an early precursor for children who subsequently develop ASD (Mitchell et al., 2006). Importantly, the lack of a significant relationship between gesture use and later non-social ALTs highlights that the association between deficits in gesture use and ALT may only be specifically related to social autistic traits/symptoms, rather than across the entire range of ALT behaviours and symptoms. This suggests that different early neurodevelopmental processes may be involved in the later emergence of social and non-social ALTs in unselected samples, thereby supporting the postulations of the Fractionable Autism Hypothesis (Happé & Ronald, 2008).

4.4.2. Imitation, play, and empathy at 12 months were not related to and did not predict later ALTs

Contrary to the study hypotheses, imitation/play abilities at 12 months were not significantly correlated with or predictive of social ALTs at 18 months, regardless of whether the three social precursors at 12 months was considered alone, or together with PPOs and temperament at 3 months. These findings are not aligned with earlier prospective studies of community samples (Bolton et al., 2012) and high-risk infant samples (Zwaigenbaum et al., 2005), which reported impairments in imitation skills and play-related behaviours within the first year of life. This difference could have arisen because of measurement limitations in assessing imitation and play in this study. Measurement of imitation and play was based on a caregiver-report measure, which is reliant on the judgment of the caregiver. In comparison, earlier studies used more comprehensive, standardized observational methods to directly and objectively assess imitation and play-related abilities in children, (Bolton et al., 2012; Zwaigenbaum et al., 2005). To the best of the author's knowledge, there is no published data regarding use of the ITSEA in Asian populations, despite it being a relatively well-established measure of early infant development in the West. Hence it is difficult to ascertain whether there may be cross-cultural differences in the interpretation of test items, which may have influenced the accuracy of findings.

A similar null association was observed for empathy at 12 months in relation to later social ALTs. In comparison with imitation and play skills, much fewer studies have examined empathy in infancy and its association with dimensionally measured ALTs. Considered together with past research

demonstrating an association between empathy and (i) ASD in infancy (i.e. Charman et al., 1997) and (ii) autistic traits in adults (Wakabayashi et al., 2007; Wheelwright et al., 2006), the study findings suggest that empathy may not function as a precursor of later ALTs during early infancy in unselected samples. Infants are typically still learning to discriminate between different facial emotions (Haviland & Lelwica, 1987; Walker-Andrews, 1998) and how to make use of emotion-related information from others to guide reciprocal affective responses (Camras & Shutter, 2010) during the first year of life. Since mastery of emotion recognition is necessary for the subsequent development of empathy-related competencies (Lombardo, Barnes, Wheelwright, & Baron-Cohen, 2007), it may be that empathy functions as a precursor of ALTs slightly later in life. Another possibility behind the null findings could be related to the method employed to assess empathy in the present study. As empathy was measured using the same scale used to assess imitation/play skills (i.e. the ITSEA), the methodological and cultural issues discussed in relation to the measurement of imitation/play in this study may apply here as well.

4.5. Study Limitations and Strengths

4.5.1. Limitations

The study findings should be interpreted in the context of a number of methodological limitations. Firstly, internal consistencies for the majority of the subscales of the EITQ and ITSEA were found to be suboptimal ($\alpha < 0.70$). These, however, were similar to the range of coefficients obtained in the original studies which evaluated the psychometric properties of these

measures (EITQ: Medoff-Cooper et al., 1993; ITSEA: Carter et al., 2003). It has been proposed that the apparent inconsistencies in accurately measuring early infant behaviours are likely due to rapid maturation during infancy, increasing exposure to the environment, and the limited range of behaviours displayed (Medoff-Cooper et al., 1993). Nevertheless, in light of growing evidence that early precursors of ALTs are observable within the first year of life, attempting to quantify early infant behaviours remains a worthwhile endeavour in spite of the inherent limitations.

The present study relied heavily on caregiver-report instruments for measuring early precursors and ALTs. The accuracy of such measures is heavily dependent on the perceptual and observational abilities of the caregivers, as well as the psychometric properties of the measures chosen. In this study, the measures employed were theoretically derived and had reasonably good psychometric properties, with the exception of low internal consistencies on some subscales. Less educated caregivers tended to report somewhat more non-social ALTs. This suggests that socio-demographic characteristics may, to some extent, influence caregiver reporting of ALTs. Other caregiver variables that could influence parental report (i.e. maternal depression, family functioning) were not considered together in this study as potential covariates. Future studies should consider incorporating more objective (observational, clinician-administered) assessment methods so as to better ensure the accuracy and ecological validity of information obtained.

In addition, respondent data was not collected for the 18-month QCHAT data. Thus, the percentage of mothers who responded at this time-point had to be estimated based on respondent data collected for measures at

other time-points. Nevertheless, it is known that the vast majority (>80%) of respondents were mothers across other caregiver measures at all time-points in the GUSTO study.

As mentioned in the study methods, a suboptimality score was used as a composite estimate of the overall risk of PPO factors in order to include variables with very low frequencies of occurrence. In addition to maximizing use of the data collected, this method is also advantageous as it accounts for possible additive effects when multiple PPO factors are present (Dodds et al., 2011). However, this method is limited in that each factor is weighted equally. Therefore, it does not account for fact that each PPO has its own unique contribution to autistic trait variability, and that some PPOs may be more strongly associated with ALTs than others.

Finally, each infant predictor of interest was examined cross-sectionally; data for each variable was collected at only one time-point. Examining trajectories of these variables by following the development of infant precursors at multiple time-points in the first and early second year of life may be more informative and reliable in identifying patterns of change over time that may be predictive of later ALTs in toddlerhood, in unselected and high-risk/clinical samples.

4.5.2. Strengths

This study also possessed a number of methodological strengths. Firstly, the study subsamples were approximately demographically representative of the full GUSTO sample which, in turn, is closely

representative of the general Singapore population (Soh et al., 2013). Hence, the study findings are likely to be generalizable to the population.

Secondly, unlike the majority of earlier investigations which used clinical or high-risk infant samples, data was prospectively collected and studied in an unselected community sample. The hypothesized relationships between infant precursors and ALTs were examined using all available participants and the entire range of scores obtained. Thus, sample selection was free from ascertainment bias and the data collected was free from memory-related distortions or hindsight bias.

Thirdly, the predictive utility of a fairly large number of early predictors was explored using a quantitative instrument designed to measure ALTs dimensionally, with some evidence of reliability and validity, rather than composite items from broad behavioural measures (e.g. Bolton et al., 2012) or categorical diagnostic measures (e.g. Zwaigenbaum et al., 2005) used in earlier studies. This allowed more reliable conclusions pertaining to the associations between early precursors and ALTs to be drawn.

Fourthly, the study explored the predictive value of a number of predictors in relation to ALTs at a much earlier time-point compared to previous studies using high-risk or clinical samples. In particular, no study has examined infant temperament as early as 3 months in relation to later ALTs. Hence, the results yielded provide preliminary evidence suggesting that infant temperament at 3 months may be predictive of ALTs within the second year of life.

Finally, this thesis examined ALTs in a non-Western population. This is important because, despite the increase in international research efforts on

ASD, few studies have sought to examine the role of culture on the perception and detection of autistic behaviours and symptoms (Kang-yi, Grinker, & Mandell, 2013). Behaviours or developmental precursors considered to be indicators of later ASD by Western researchers can be perceived and reported differently in Asian cultures (Soto et al., 2014). For example, the American Academy of Pediatrics (AAP) identifies a range of possible indicators of later autistic symptoms in toddlers, one such indicator being lack of eye contact (Johnson & Myers, 2007). However, parents from China or Japan are less likely to perceive this as a possible indicator of ASD, since the absence of direct eye contact is considered to be a desirable display of humility or respect in East Asia (Le Roux, 2002). Clinicians in India are less likely to refer male toddlers with delayed communication abilities for diagnostic evaluation, owing to a cultural belief that male toddlers acquire speech later than their female counterparts (Daley & Sigman, 2002). In Korean culture, the presence of autistic-like behaviours, even if detected, could be attributed to other causes such as poor prenatal care or parenting, rather than the possible presence of ASD (Cho, Singer, & Brenner, 2000). In addition, ALTs were assessed in this study using a measure developed for use in a Western population. Given that existing measures of ALTs have mostly been developed in Western contexts, it is important to study the use of these instruments in non-Western populations to facilitate cross-cultural comparisons, so as to identify possible inter-cultural differences in the ways in which test items may be interpreted and scored by parents in other cultures. In this study, the mean distribution of ALT scores was found to be higher than those reported by caregivers in Western-based samples of children aged 18 to 24 months (e.g. Allison et al.,

2008; Auyeung et al., 2010), indicating that caregiver interpretation of ALTs may indeed be influenced by cultural factors to some degree. However, internal consistency was good for the factor scores and the ALT score distribution was approximately normal, as in most other studies of autistic traits in Western samples. Nevertheless, this reinforces the importance of the cultural adaptation of Western-developed tools for use in other populations.

4.6. Implications of the study's findings and contributions to the existing literature

The present thesis is, to the author's knowledge, the first to examine the predictive value of a number of early infancy precursors on autistic trait variability at 18 months, using a validated measure of ALTs designed for use in unselected populations. Importantly, this study is also the first to investigate the predictive utility of early precursors separately for social and non-social ALTs, rather than for overall/total ALTs.

The results of this study extend previous research findings from twin cohorts in population-based samples (i.e. Robinson et al., 2012; Ronald et al., 2005, 2006a), which provided preliminary evidence that the different core autistic symptoms are 'fractionable' in terms of their specific etiological causes. In this study, there was little overlap between individual infant precursors that were associated with and predictive of social ALTs, relative to that for non-social ALTs. This resonates with earlier findings that different sets of genetic and environmental likely underpin different clusters of autistic traits Robinson et al., 2012; Ronald et al., 2005, 2006a). Therefore, the results of this study largely support the fractionality of ALTs.

The study findings are aligned with earlier factor analytic studies, suggesting that the overall autism construct is not comprised of a single underlying factor, but rather, composed of distinct clusters of autistic-like behaviours (Shuster et al., 2014). In this study, social and non-social ALTs were significantly correlated with overall ALTs with approximately large effect sizes (r = .48, r = .70 respectively; both ps < .001). However, they were only modestly correlated with each other, and there was little overlap in the precursors associated with each group of ALTs. Importantly, this provides further evidence that the dyadic symptom model for ASD proposed in the DSM-5 (American Psychiatric Association, 2013; social communication and RRBIs) may also be applicable for organizing ALTs in the general population, across the continuum of autism symptoms.

Importantly, this study provides some preliminary evidence that some of the early factors reported in earlier clinical studies to be predictive of later ASD diagnosis, and which contribute to explaining autistic symptoms at the extreme end of the continuum, are also relevant and predict ALTs across the whole range of scores/symptoms in unselected participants from the general population. Specifically temperament and gestures, reported in earlier literature as predicting ASD risk (see Introduction, sections 1.5 and 1.6), were also found to be significant predictors of ALTs in our unselected sample. However, PPOs was not found to be a significant predictor of social or non-social ALTs, despite demonstrating an association with higher risk of ASD in earlier studies (see Introduction, section 1.4). Although this could be due to limitations in the way PPO suboptimality scores were measured in this study, this finding points towards PPOs' potentially unique role in possibly

explaining autistic trait variability only at the extreme ends of the ALT continuum. This hypothesis, however, needs to be replicated in studies using much larger unselected samples, with a sufficiently large number of individuals falling within the quantitative extreme range (defined as top 5% in most studies; see Robinson et al., 2011). This is important since it has been suggested that the influence of PPOs may be more salient in individuals with higher genetic susceptibility, owing to the role of gene-environment interactions in contributing to autistic traits/symptoms (Lundstrom et al 2012; Ronald & Hoekstra, 2010; Sandin et al., 2013).

Finally, this study provides some cross-cultural support for the potential importance of a number of early infancy variables as predictors of ALTs. To the best of the author's knowledge, no non-Western study has explored whether this extends to dimensionally measured ALTs in children younger than 2 years of age. Despite possible cultural differences in the interpretation and scoring of Q-CHAT items, it was found that temperament at 3 months and gestures at 12 months significantly predicted later ALTs at 18 months. This provides some evidence of the cross-cultural stability of the relationships between early infancy precursors and later ALTs. Proper cultural adaptation of the test items of Western-based dimensional measures of ALT and replication on other Asian samples by future studies may be useful for confirming the present findings.

The study findings may also have implications for future research aiming to elucidate the role of early developmental factors on early neurodevelopment in ASD. The fact that social and non-social ALTs appear to contribute independently to overall autistic trait variability and are associated

with different infant precursors suggests that they are likely the result of complex, but possibly different, neurodevelopmental processes. This, in turn, indicates that the two clusters of traits are likely underpinned by different sets of etiological factors. By studying infant precursors of social and non-social ALTs in toddlerhood, this study extended previous work on the fractionable nature of ALTs conducted in twin samples in middle to late childhood (7-12 years; Ronald et al., 2005, 2006a, 2006b, Robinson et al., 2012). Hence, these findings echo Ronald and Hoekstra's (2014) recommendations that future research examining the causes of ASD need necessarily study hypothesized causal factors in relation to individual autistic trait dimensions.

4.7. Recommendations for Future Research

The present study has highlighted four areas which merit consideration by future empirical efforts. Firstly, the results obtained in this unselected community sample are similar to the findings of earlier population-based twin studies in that they both support the hypothesis that the etiological influences of ASD/ALTs are likely 'fractionable' (Happé & Ronald, 2008). Thus, future research seeking to elucidate the causes or early developmental pathways leading to ASD/ALTs should therefore examine these variables separately in relation to the two different clusters of autistic symptoms/traits, rather than in relation to overall autistic trait severity as has been done by earlier studies using clinical or high-risk samples.

Secondly, future research should employ prospective, longitudinal designs for examining the predictive utility of early infancy precursors on different clusters of ALTs. In particular, no study has examined temperament

as early as 3 months of age, in relation to later ALTs. However, infant behaviours and competencies are likely to change as a result of rapid maturation and progressively greater exposure to the environment across infancy and toddlerhood (Medoff-Cooper et al., 1993). In order to confirm the stability of the associations found in the present study, examining developmental trajectories of early precursors on later social and non-social ALTs may provide a more reliable means for gaining insight into the neurodevelopmental pathways implicated in social versus non-social ALTs, as well as for identifying infants who are at high risk.

Thirdly, as research on the genetic underpinnings of ASDs/ALTs uncovers more reliable and specific genetic risk factors, incorporating these factors in future study of early markers of ALTs may be informative for advancing inquiry into the neurobiological impact of these causative influences. This is important since genetic factors have been reported to account for close to 50% of the variability in ALTs in children as young as two years of age from the general population (Edelson & Saudino, 2009). Furthermore, it has been suggested that there may be a positive relationship between genetic vulnerability and birth/obstetric suboptimality (Sandin et al., 2013). Thus, studying specific genetic risk factors together with PPO risk factors may help to explicate the nature of gene-PPO interactions and later ALTs (Glasson et al., 2004), as well as to delineate the independent contributions of PPO factors in relation to the risk of ASD, or variability in ALTs (Sandin et al., 2013).

Finally, as highlighted earlier, few studies have examined the role of early precursors on later ALTs in non-Western contexts. Thus, future

replications on other unselected community samples from non-Western cultures are required so as to better determine the cross-cultural stability of associations between early infancy precursors and ALTs in toddlerhood. Comparison of findings between multiple non-Western studies is essential for ascertaining the degree to which the results yielded in this study can be generalized to other ethnically/culturally similar populations, as well as for facilitating future cross-cultural comparisons between Western and non-Western studies.

4.8. Summary and Conclusions

The findings of this thesis showed that different infant precursors in the first year of life predicted social and non-social ALTs at 18 months. Different temperament dimensions at 3 months were associated with and predicted social vs. non-social ALTs at 18 months. Gesture use at 12 months predicted only social ALTs at 18 months. Pregnancy and birth-related risk factors implicated in ASD, and imitation and empathy at 12 months were neither significantly associated with nor predictive of social and non-social ALTs. Considered together with findings from population-based twin studies, this thesis provides further evidence and cross-cultural support suggesting that the etiological contributions and neurobiological abnormalities underpinning the different core autistic dimensions are likely different, and that a number of infant predictors are shared across the whole range of ALTs.

References

Abrahams, B. S., & Geschwind, D. H. (2008). Advances in autism genetics: on the threshold of a new neurobiology. *Nature Reviews Genetics, 9*(5), 341-355. doi: 10.1038/nrg2346

Adrien, J. L., Lenoir, P., Martineau, J., Perrot, A., Hameury, L., Larmande, C., & Sauvage, D. (1993). Blind ratings of early symptoms of Autism based upon family home movies. *Journal of the American Academy of Child and Adolescent Psychiatry, 32*(3), 617-626.

Allison, C., Baron-Cohen, S., Wheelwright, S., Charman, T., Richler, J., Pasco, G., & Brayne, C. (2008). The Q-CHAT (Quantitative Checklist for Autism in Toddlers): a normally distributed quantitative measure of autistic traits at 18-24 months of age: preliminary report. *Journal of Autism and Developmental Disorders*, *38,* 1414 - 1425. doi: doi: 10.1007/s10803-007-0509-7

Allison, C., Pasco, G., Wheelwright, S.J., Charman, T., Brayne, C., & Baron-Cohen, S. (2010, May 22). *Factor structure of the Q-CHAT, a revised screening instrument for Autism Spectrum Conditions in toddlers between 18 – 24 months.* Poster presented at the International Meeting for Autism Research, Philadelphia, PA.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). doi:10.1176/appi.books.9780890423349.

American Psychiatric Association. (2013). *The Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Annibale, D. J., Hulsey, T. C., Wagner, C. L., & Southgate, W. (1995). Comparative neonatal morbidity of abdominal and vaginal deliveries after uncomplicated pregnancies. *Archives of Pediatrics & Adolescent Medicine, 149*(8), 862-867. doi: 10.1001/archpedi.1995.02170210036006

Aronson, M., Hagberg, B., & Gillberg, C. (1997). Attention deficits and autistic spectrum problems in children exposed to alcohol during gestation: a follow-up study. *Developmental Medicine & Child Neurology, 39*(9), 583-587. doi: 10.1111/j.1469-8749.1997.tb07493.x

Auyeung, B., Baron-Cohen, S., Chapman, E., Knickmeyer, R., Taylor, K., & Hackett, G. (2009). Fetal testosterone and autistic traits. *British Journal of Psychology, 100*, 1 - 22.

Auyeung, B., Taylor, K., Hackett, G., & Baron-Cohen, S. (2010). Foetal testosterone and autistic traits in 18 to 24-month-old children. *Molecular Autism, 1*(1), 11. doi: 10.1186/2040-2392-1-11

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., & Rutter, M. (1995). Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine, 25*(01), 63-77. doi: doi:10.1017/S0033291700028099

Baird, G., Charman, T., Baron-Cohen, S., Cox, A., Swettenham, J., Wheelwright, S., & Drew, A. (2000). A screening instrument for Autism at 18 months of age: a 6-Year follow-up study. *Journal of the American Academy of Child & Adolescent Psychiatry, 39*(6), 694-702. doi: http://dx.doi.org/10.1097/00004583-200006000-00007

Bakeman, R., & Adamson, L. B. (1984). Coordinating attention to people and objects in mother-infant and peer-infant interaction. *Child Development, 55*(4), 1278-1289.

Baranek, G. (1999). Autism During Infancy: A retrospective video analysis of sensory-motor and social behaviors at 9–12 months of age. *Journal of Autism and Developmental Disorders, 29*(3), 213-224. doi: 10.1023/a:1023080005650

Baranek, G. T., Boyd, B. A., Poe, M. D., David, F. J., Watson, L. R., & MacLean Jr, W. E. (2007). Hyperresponsive sensory patterns in young children with autism, developmental delay, and typical development. *American Journal on Mental Retardation, 112*(4), 233-245. doi: 10.1352/0895-8017(2007)112[233:hspiyc]2.0.co;2

Baron-Cohen, S., Allen, J., & Gillberg, C. (1992). Can autism be detected at 18 months? The needle, the haystack, and the CHAT. *The British Journal of Psychiatry, 161*(6), 839-843. doi: 10.1192/bjp.161.6.839

Baron-Cohen, S., & Wheelwright, S. (2004). The Empathy Quotient: an investigation of adults with Asperger Syndrome or High Functioning Autism, and normal sex differences. *Journal of Autism and Developmental Disorders, 34*(2), 163-175. doi: 10.1023/b:jadd.0000022607.19833.00

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The Autism Spectrum Quotient (AQ): evidence from Asperger syndrome/high functioning autism, males and females, scientists and mathematicians. *Journal of Autism and Developmental Disorders, 31*, 5 - 17.

Bilder, D., Pinborough-Zimmerman, J., Miller, J., & McMahon, W. (2009). Prenatal, perinatal, and neonatal factors associated sith Autism Spectrum Disorders. *Pediatrics, 123*(5), 1293-1300. doi: 10.1542/peds.2008-0927

Bishop, D. V. M., Maybery, M., Wong, D., Maley, A., & Hallmayer, J. (2006). Characteristics of the broader phenotype in autism: A study of siblings using the children's communication checklist-2. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 141B*(2), 117-122. doi: 10.1002/ajmg.b.30267

Bolton, P., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., . . . Rutter, M. (1994). A case-control family history study of Autism. *Journal of Child Psychology and Psychiatry, 35*(5), 877-900. doi: 10.1111/j.1469-7610.1994.tb02300.x

Bolton, P. F., Golding, J., Emond, A., & Steer, C. D. (2012). Autism Spectrum Disorder and autistic traits in the Avon Longitudinal Study of Parents and Children: precursors and early signs. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(3), 249-260.e225. doi: http://dx.doi.org/10.1016/j.jaac.2011.12.009

Bolton, P. F., Murphy, M., Macdonald, H., Whitlock, B., Pickles, A., & Rutter, M. (1997). Obstetric complications in Autism: consequences or causes of the condition? *Journal of the American Academy of Child & Adolescent Psychiatry, 36*(2), 272-281. doi: http://dx.doi.org/10.1097/00004583-199702000-00018

Bolton, P. F., Pickles, A., Murphy, M., & Rutter, M. (1998). Autism, affective and other psychiatric disorders: patterns of familial aggregation. *Psychological Medicine, 28*(02), 385-395. doi: doi:null

Bora, E., Gökçen, S., & Veznedaroglu, B. (2008). Empathic abilities in people with schizophrenia. *Psychiatry Research, 160*(1), 23-29.

Briggs-Gowan, M. J., & Carter, A. S. (1998). Preliminary acceptability and psychometrics of the infant–toddler social and emotional assessment (ITSEA): A new adult-report questionnaire. *Infant Mental Health Journal, 19*(4), 422-445. doi: 10.1002/(sici)1097-0355(199824)19:4<422::aid-imhj5>3.0.co;2-u

Brock, M. E., Freuler, A., Baranek, G. T., Watson, L. R., Poe, M. D., & Sabatino, A. (2012). Temperament and sensory features of children with Autism. *Journal of Autism and Developmental Disorders, 42*(11), 2271-2284. doi: 10.1007/s10803-012-1472-5

Bryson, S. E., Smith, I. M., & Eastwood, D. (1988). Obstetrical suboptimality in autistic children. *Journal of the American Academy of Child & Adolescent Psychiatry, 27*(4), 418-422.

Buchmayer, S., Johansson, S., Johansson, A., Hultman, C. M., Sparén, P., & Cnattingius, S. (2009). Can association between preterm birth and Autism be explained by maternal or neonatal morbidity? *Pediatrics, 124*(5), e817-e825. doi: 10.1542/peds.2008-3582

Burstyn, I., Sithole, F., & Zwaigenbaum, L. (2010). Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta, Canada. *Chronic Diseases in Canada, 30*(4), 125-134.

Camras, L. A., & Shutter, J. M. (2010). Emotional facial expressions in infancy. *Emotion Review, 2*(2), 120-129. doi: 10.1177/1754073909352529

Carey, W. B., & McDevitt, S. C. (1995). *The Carey temperament scales*. Scottsdale, AZ: Behavioral-Developmental Initiatives.

Carey, W. (2007). *The Carey Temperament Scales Test Manual (2nd ed.).* Arizona: Behavioral-Developmental Initiatives.

Carter, A., Briggs-Gowan, M., Jones, S., & Little, T. (2003). The Infant–Toddler Social and Emotional Assessment (ITSEA): factor structure, reliability, and validity. *Journal of Abnormal Child Psychology, 31*(5), 495-514. doi: 10.1023/a:1025449031360

Carter, A. S., & Briggs-Gowan, M. J. (2006). ITSEA infant-toddler social and emotional assessment examiner's manual. *San Antonio, TX: PsychCorp*.

Cavell, T. A. (1990). Social adjustment, social performance, and social skills: a tri-component model of social competence. *Journal of Clinical Child Psychology, 19*(2), 111-122. doi: 10.1207/s15374424jccp1902_2

Chakrabarti, S., & Fombonne, E. (2001). Pervasive developmental disorders in preschool children. *JAMA, 285*(24), 3093-3099. doi: 10.1001/jama.285.24.3093

Charman, T., Swettenham, J., Baron-Cohen, S., Cox, A., Baird, G., & Drew, A. (1997). Infants with autism: an investigation of empathy, pretend play, joint attention, and imitation. *Developmental psychology, 33*(5), 781-789. doi: 10.1037/0012-1649.33.5.781

Christensen, L., Hutman, T., Rozga, A., Young, G., Ozonoff, S., Rogers, S., . . . Sigman, M. (2010). Play and developmental outcomes in infant siblings of children with Autism. *Journal of Autism and Developmental Disorders, 40*(8), 946-957. doi: 10.1007/s10803-010-0941-y

Cho, S., Singer, G. H. S., & Brenner, M. (2000). Adaptation and accommodation to young children with disabilities: A comparison of Korean and Korean American parents. *Topics in Early Childhood Special Education, 20*(4), 236–249.

Cnattingius, S. (2004). The epidemiology of smoking during pregnancy: Smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine & Tobacco Research, 6*(Suppl 2), S125-S140. doi: 10.1080/14622200410001669187

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates, Inc.

Constantino, J. N., Lajonchere, C., Lutz, M., Gray, T., Abbacchi, A., McKenna, K., . . . Todd, R. D. (2006). Autistic social impairment in the siblings of children with Pervasive Developmental Disorders. *American Journal of Psychiatry, 163*(2), 294-296.

Constantino, J. N., & Todd, R. D. (2003). Autistic traits in the general population: A twin study. *Archives of General Psychiatry, 60*(5), 524-530. doi: 10.1001/archpsyc.60.5.524

Croen, L. A., Najjar, D. V., Fireman, B., & Grether, J. K. (2007). Maternal and paternal age and risk of autism spectrum disorders. *Archives of*

Pediatrics & Adolescent Medicine, 161(4), 334-340. doi: 10.1001/archpedi.161.4.334

Daley, T. C., & Sigman, M. D. (2002). Diagnostic conceptualization of autism among Indian psychiatrists, psychologists, and pediatricians. *Journal of Autism and Developmental disorders, 32*(1), 13-23.

Davis, M. H. (1994). *Empathy: A social psychological approach*. Boulder, CO: Westview Press.

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., & Liaw, J. (2004). *Developmental psychology, 40*(2), 271-283. doi: 10.1037/0012-1649.40.2.271

Dawson, G., Webb, S., Schellenberg, G. D., Dager, S., Friedman, S., Aylward, E., & Richards, T. (2002). Defining the broader phenotype of autism: Genetic, brain, and behavioral perspectives. *Development and Psychopathology, 14*(03), 581-611. doi: doi:10.1017/S0954579402003103

De Giacomo, A., & Fombonne, E. (1998). Parental recognition of developmental abnormalities in autism. *European Child & Adolescent Psychiatry, 7*(3), 131-136. doi: 10.1007/s007870050058

del Rosario, M., Gillespie-Lynch, K., Johnson, S., Sigman, M., & Hutman, T. (2014). Parent-reported temperament trajectories among infant siblings of children with Autism. *Journal of Autism and Developmental Disorders, 44*(2), 381-393. doi: 10.1007/s10803-013-1876-x

Dodds, L., Fell, D., Shea, S., Armson, B. A., Allen, A., & Bryson, S. (2011). The role of prenatal, obstetric and neonatal factors in the development

of Autism. *Journal of Autism and Developmental Disorders, 41*(7), 891-902. doi: 10.1007/s10803-010-1114-8

Eaton, W., Mortensen, P., Thomsen, P., & Frydenberg, M. (2001). Obstetric complications and risk for severe psychopathology in childhood. *Journal of Autism and Developmental Disorders, 31*(3), 279-285. doi: 10.1023/a:1010743203048

Edelson, L. R., & Saudino, K. J. (2009). Genetic and environmental influences on Autistic-like behaviors in 2-year-old twins. *Behavior Genetics, 39*(3), 255-264. doi: 10.1007/s10519-009-9270-3

Eliasen, M., Tolstrup, J. S., Nybo Andersen, A.-M., Grønbæk, M., Olsen, J., & Strandberg-Larsen, K. (2010). Prenatal alcohol exposure and autistic spectrum disorders—a population-based prospective study of 80 552 children and their mothers. *International Journal of Epidemiology*. doi: 10.1093/ije/dyq056

Fenson, L., Dale, P. S., Reznick, J. S., Bates, E., Thal, D. J., Pethick, S. J., . . . Stiles, J. (1994). Variability in early communicative development. *Monographs of the Society for Research in Child Development, 59*(5), v-173. doi: 10.2307/1166093

Field, A. (2009). *Discovering statistics using SPSS* (3rd ed.). London, UK: Sage publications.

Folstein, S., & Rutter, M. (1977). Infantile autism: a genetic study of 21 twin pairs. *Journal of Child Psychology and Psychiatry, 18*(4), 297-321. doi: 10.1111/j.1469-7610.1977.tb00443.x

Fombonne, E. (2003). Epidemiological surveys of Autism and other Pervasive Developmental Disorders: An Update. *Journal of Autism and*

Developmental Disorders, 33(4), 365-382. doi: 10.1023/a:1025054610557

Fombonne, E. (2005). The changing epidemiology of Autism. *Journal of Applied Research in Intellectual Disabilities, 18*(4), 281-294. doi: 10.1111/j.1468-3148.2005.00266.x

Fombonne, E. (2006). Past and future perspectives on autism epidemiology. In: Understanding autism, from basic neuroscience to treatment. (pp. 25-48): Boca Raton, FL, USA: Taylor & Francis.

Frazier, T., Youngstrom, E., Kubu, C., Sinclair, L., & Rezai, A. (2008). Exploratory and Confirmatory Factor Analysis of the Autism Diagnostic Interview-Revised. *Journal of Autism and Developmental Disorders, 38*(3), 474-480. doi: 10.1007/s10803-007-0415-z

Freeth, M., Sheppard, E., Ramachandran, R., & Milne, E. (2013). A cross-cultural comparison of autistic traits in the UK, India and Malaysia. *Journal of Autism and Developmental Disorders, 43*(11), 2569-2583. doi: 10.1007/s10803-013-1808-9

Gardener, H., Spiegelman, D., & Buka, S. L. (2009). Prenatal risk factors for autism: comprehensive meta-analysis. *The British Journal of Psychiatry, 195*(1), 7-14. doi: 10.1192/bjp.bp.108.051672

Gardener, H., Spiegelman, D., & Buka, S. L. (2011). Perinatal and Neonatal Risk Factors for Autism: A Comprehensive Meta-analysis. *Pediatrics, 128*(2), 344-355. doi: 10.1542/peds.2010-1036

Garon, N., Bryson, S., Zwaigenbaum, L., Smith, I., Brian, J., Roberts, W., & Szatmari, P. (2009). Temperament and its Relationship to Autistic

Symptoms in a High-Risk Infant Sib Cohort. *Journal of Abnormal Child Psychology, 37*(1), 59-78. doi: 10.1007/s10802-008-9258-0

Gartstein, M. A., & Rothbart, M. K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development, 26*(1), 64-86. doi: http://dx.doi.org/10.1016/S0163-6383(02)00169-8

Gerdts, J., & Bernier, R. (2011). The Broader Autism Phenotype and its implications on the etiology and treatment of Autism Spectrum Disorders. *Autism Research and Treatment, vol. 2011*(545901). doi: 10.1155/2011/545901

Gest, S., Sesma, A., Jr., Masten, A., & Tellegen, A. (2006). Childhood peer reputation as a predictor of competence and symptoms 10 Years later. *Journal of Abnormal Child Psychology, 34*(4), 507-524. doi: 10.1007/s10802-006-9029-8

Gillberg, C., & Gillberg, I. C. (1983). Infantile autism: A total population study of reduced optimality in the pre-, peri-, and neonatal period. *Journal of Autism and Developmental Disorders, 13*(2), 153-166. doi: 10.1007/bf01531816

Ginsburg, C., Fokstuen, S., & Schinzel, A. (2000). The contribution of uniparental disomy to congenital development defects in children born to mothers at advanced childbearing age. *American Journal of Medical Genetics, 95*(5), 454-460. doi: 10.1002/1096-8628(20001218)95:5<454::aid-ajmg9>3.0.co;2-o

Glasson, E. J., Bower, C., Petterson, B., de Klerk, N., Chaney, G., & Hallmayer, J. F. (2004). Perinatal factors and the development of

autism: A population study. *Archives of General Psychiatry, 61*(6), 618-627. doi: 10.1001/archpsyc.61.6.618

Goldberg, W., Jarvis, K., Osann, K., Laulhere, T., Straub, C., Thomas, E., . . . Spence, M. A. (2005). Brief report: early social communication behaviors in the younger siblings of children with Autism. *Journal of Autism and Developmental Disorders, 35*(5), 657-664. doi: 10.1007/s10803-005-0009-6

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. *Journal of Autism and Developmental Disorders, 37*(4), 613-627. doi: 10.1007/s10803-006-0280-1

Guinchat, V., Thorsen, P., Laurent, C., Cans, C., Bodeau, N., & Cohen, D. (2012). Pre-, peri- and neonatal risk factors for autism. *Acta Obstetricia et Gynecologica Scandinavica, 91*(3), 287-300. doi: 10.1111/j.1600-0412.2011.01325.x

Happé, F., & Ronald, A. (2008). The 'Fractionable Autism Triad': A review of evidence from behavioural, genetic, cognitive and neural research. *Neuropsychology Review, 18*(4), 287-304. doi: 10.1007/s11065-008-9076-8

Happé, F., Ronald, A., & Plomin, R. (2006). Time to give up on a single explanation for autism. *Nature Neuroscience, 9*(10), 1218-1220. doi: 10.1038/nn1770

Harris, S. R., MacKay, L. L. J., & Osborn, J. A. (1995). Autistic behaviors in offspring of mothers abusing alcohol and other drugs: a series of case

reports. *Alcoholism: Clinical and Experimental Research, 19*(3), 660-665. doi: 10.1111/j.1530-0277.1995.tb01564.x

Haviland, J. M., & Lelwica, M. (1987). The induced affect response: 10-week-old infants' responses to three emotion expressions. *Developmental psychology, 23*(1), 97.

Hepburn, S., & Stone, W. (2006). Using Carey Temperament Scales to assess behavioral style in children with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders, 36*(5), 637-642. doi: 10.1007/s10803-006-0110-5

Hobson, R. P. (1993). *Autism and the development of mind*. London: Erlbaum.

Hoekstra, R., Bartels, M., Cath, D., & Boomsma, D. (2008). Factor structure, reliability and criterion validity of the Autism-spectrum Quotient (AQ): a study in Dutch population and patient groups. *Journal of Autism and Developmental Disorders, 38*, 1555 - 1566.

Hofheimer, J. A., Sheinkopf, S. J., & Eyler, L. T. (2014). Autism risk in very preterm infants: new answers, more questions. *The Journal of Pediatrics, 164*(1), 6 - 8.

Hoshino, Y., Kaneko, M., Yashima, Y., Kumashiro, H., Volkmar, F. R., & Cohen, D. J. (1987). Clinical Features of Autistic Children with Setback Course in Their Infancy. *Psychiatry and Clinical Neurosciences, 41*(2), 237-245. doi: 10.1111/j.1440-1819.1987.tb00407.x

Hudziak, J. J., Achenbach, T. M., Althoff, R. R., & Pine, D. S. (2007). A dimensional approach to developmental psychopathology.

International Journal of Methods in Psychiatric Research, 16(S1), S16-S23. doi: 10.1002/mpr.217

Hultman, C. M., Sparén, P., & Cnattingius, S. (2002). Perinatal Risk Factors for Infantile Autism. *Epidemiology, 13*(4), 417-423.

Hurley, R. E., Losh, M., Parlier, M., Reznick, J. S., & Piven, J. (2007). The Broad Autism Phenotype Questionnaire. *Journal of Autism and Developmental Disorders, 37*(9), 1679-1690. doi: 10.1007/s10803-006-0299-3

Ibañez, L. V., Grantz, C. J., & Messinger, D. S. (2013). The development of referential communication and Autism symptomatology in high-risk infants. *Infancy, 18*(5), 687-707. doi: 10.1111/j.1532-7078.2012.00142.x

Ikonomidou, C., Bittigau, P., Ishimaru, M. J., Wozniak, D. F., Koch, C., Genz, K., . . . Olney, J. W. (2000). Ethanol-induced apoptotic neurodegeneration and Fetal Alcohol Syndrome. *Science, 287*(5455), 1056-1060. doi: 10.1126/science.287.5455.1056

Johnson, C. P., & Myers, S. M. (2007). Identification and evaluation of children with autism spectrum disorders. *Pediatrics, 120*(5), 1183-1215.

Jones, M., & Szatmari, P. (1988). Stoppage rules and genetic studies of autism. *Journal of Autism and Developmental Disorders, 18*(1), 31-40. doi: 10.1007/bf02211816

Juul-Dam, N., Townsend, J., & Courchesne, E. (2001). Prenatal, perinatal, and neonatal Factors in Autism, Pervasive Developmental Disorder-Not

Otherwise Specified, and the general population. *Pediatrics, 107*(4), e63.

Kalkbrenner, A. E., Braun, J. M., Durkin, M. S., Maenner, M. J., Cunniff, C., Lee, L.-C., . . . Daniels, J. L. (2012). Maternal smoking during pregnancy and the prevalence of autism spectrum disorders, using data from the Autism and Developmental Disabilities Monitoring Network. *Environmental Health Perspectives, 120*(7), 1042. doi: 10.1289/ehp.1104556

Kang-Yi, C. D., Grinker, R. R., & Mandell, D. S. (2013). Korean culture and autism spectrum disorders. *Journal of Autism and Developmental Disorders, 43*(3), 503-520.

Kanne, S. M., Christ, S. E., & Reiersen, A. M. (2009). Psychiatric symptoms and psychosocial difficulties in young adults with autistic traits. *Journal of Autism and Developmental Disorders, 39*(6), 827-833. doi: 10.1007/s10803-008-0688-x

Kim, H.-Y. (2013). Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis. *Restorative dentistry & endodontics, 38*(1), 52-54.

Klauck, S. M. (2006). Genetics of autism spectrum disorder. *European Journal of Human Genetics, 14*(6), 714-720. doi: 10.1038/sj.ejhg.5201610

Kolevzon, A., Gross, R., & Reichenberg, A. (2007). Prenatal and perinatal risk factors for autism: A review and integration of findings. *Archives of Pediatrics & Adolescent Medicine, 161*(4), 326-333. doi: 10.1001/archpedi.161.4.326

Kuehn, B. M. (2007). Cdc: Autism spectrum disorders common. *JAMA, 297*(9), 940-940. doi: 10.1001/jama.297.9.940

Landa, R. (2007). Early communication development and intervention for children with autism. *Mental Retardation and Developmental Disabilities Research Reviews, 13*(1), 16-25. doi: 10.1002/mrdd.20134

Larsson, H. J., Eaton, W. W., Madsen, K. M., Vestergaard, M., Olesen, A. V., Agerbo, E., . . . Mortensen, P. B. (2005). Risk factors for Autism: perinatal Factors, parental psychiatric history, and socioeconomic status. *American Journal of Epidemiology, 161*(10), 916-925. doi: 10.1093/aje/kwi123

Lauritsen, M., & Ewald, H. (2001). The genetics of autism. *Acta Psychiatrica Scandinavica, 103*(6), 411-427. doi: 10.1034/j.1600-0447.2001.00086.x

Lee, B., Gardner, R., Dal, H., Svensson, A., Galanti, M., Rai, D., . . . Magnusson, C. (2012). Brief report: maternal smoking during pregnancy and Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders, 42*(9), 2000-2005. doi: 10.1007/s10803-011-1425-4

Le Roux, J. (2002). Effective educators are culturally competent communicators. Intercultural Education, 13(1), 37-48.

Leyfer, O. T., Folstein, S. E., Bacalman, S., Davis, N. O., Dinh, E., Morgan, J., . . . Lainhart, J. E. (2006). Comorbid psychiatric disorders in children with Autism: interview development and rates of disorders. *Journal of Autism and Developmental Disorders, 36*(7), 849-861. doi: 10.1007/s10803-006-0123-0

Lombardo, M. V., Barnes, J. L., Wheelwright, S. J., & Baron-Cohen, S. (2007). Self-referential cognition and empathy in autism. *PloS one, 2*(9), e883. doi: doi:10.1371/journal.pone.0000883

Lord, C., Mulloy, C., Wendelboe, M., & Schopler, E. (1991). Pre- and perinatal factors in high-functioning females and males with autism. *Journal of Autism and Developmental Disorders, 21*(2), 197-209. doi: 10.1007/bf02284760

Lord, C., Risi, S., Lambrecht, L., Cook, E., Jr., Leventhal, B., DiLavore, P., . . . Rutter, M. (2000). 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, 30*(3), 205-223. doi: 10.1023/a:1005592401947

Lord, C., Rutter, M., & Couteur, A. (1994). Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*(5), 659-685. doi: 10.1007/bf02172145

Lord, C., Rutter, M., DiLavore, P., & Risi, S. (1999). *Autism Diagnostic Observation Schedule (ADOS) manual*. Los Angeles, CA: Western Psychological Services

Low, E. L., & Brown, A. (2005). *English in Singapore: an introduction*. Singapore: McGraw-Hill.

Lundström, S., Chang, Z., Råstam, M., & et al. (2012). Autism spectrum disorders and autisticlike traits: Similar etiology in the extreme end and

the normal variation. *Archives of General Psychiatry, 69*(1), 46-52. doi: 10.1001/archgenpsychiatry.2011.144

Madan, A., Holland, S., Humbert, J. E., & Benitz, W. E. (2001). Racial differences in birth weight of term infants in a northern California population. *Journal of perinatology: official journal of the California Perinatal Association, 22*(3), 230-235.

Magiati, I., Goh, D.A., Lim, S.J.J., Leong, C.L.J., Rifkin-Graboi, A.,Leutscher-Broekman, B., Meaney, M., Allison, C., Baron-Cohen, S. & the GUSTO working group (in preparation). *The psychometric properties of the Quantitative-Checklist for Autism in Toddlers (Q-CHAT) as a measure of autistic traits in a community sample of Singaporean pre-school children.*

Maimburg, R. D., & Væth, M. (2006). Perinatal risk factors and infantile autism. *Acta Psychiatrica Scandinavica, 114*(4), 257-264. doi: 10.1111/j.1600-0447.2006.00805.x

Mandy, W. P. L., Charman, T., & Skuse, D. H. (2012). Testing the construct validity of proposed criteria for DSM-5 Autism Spectrum Disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(1), 41-50.

Mandy, W. P. L., & Skuse, D. H. (2008). Research Review: What is the association between the social-communication element of autism and repetitive interests, behaviours and activities? *Journal of Child Psychology and Psychiatry, 49*(8), 795-808. doi: 10.1111/j.1469-7610.2008.01911.x

Mars, A. E., Mauk, J. E., & Dowrick, P. W. (1998). Symptoms of pervasive developmental disorders as observed in prediagnostic home videos of infants and toddlers. *The Journal of Pediatrics, 132*(3), 500-504. doi: http://dx.doi.org/10.1016/S0022-3476(98)70027-7

Matson, J. L., Boisjoli, J. A., Hess, J. A., & Wilkins, J. (2010). Factor structure and diagnostic fidelity of the Baby and Infant Screen for Children with aUtIsm Traits–Part 1 (BISCUIT–part 1). *Developmental Neurorehabilitation, 13*(2), 72-79. doi: doi:10.3109/17518420903213576

Matson, J. L., Worley, J. A., Fodstad, J. C., Chung, K. M., Suh, D., Jhin, H. K., ... & Furniss, F. (2011). A multinational study examining the cross cultural differences in reported symptoms of autism spectrum disorders: Israel, South Korea, the United Kingdom, and the United States of America. *Research in Autism Spectrum Disorders, 5*(4), 1598-1604.

McDevitt, S. C., & Carey, W. B. (1978). The measurement of temperament in 3-7 year old children. *Journal of Child Psychology and Psychiatry, 19*(3), 245-253. doi: 10.1111/j.1469-7610.1978.tb00467.x

Mealey, L., & Kinner, S. (2002). The perception-action model of empathy and psychopathic "cold-heartedness". *Behavioral and Brain Sciences, 25*(1), 42-43.

Medoff-Cooper, B., Carey, W. B., & McDevitt, S. C. (1993). The Early Infancy Temperament Questionnaire. *Journal of Developmental and Behavioral Pediatrics, 14*(4), 230-235.

Mervielde, I., & De Pauw, S. S. W. (2012). Models of child temperament. In

M. Zentner & R. L. Shiner (Eds.), Handbook of temperament (1st ed., pp. 21–40). NY: The Guilford Press.

Meek, S. E., Lemery-Chalfant, K., Jahromi, L. B., & Valiente, C. (2013). A review of gene-environment correlations and their implications for autism: a conceptual model. *Psychological review, 120*(3), 497-521. doi: doi: 10.1037/a0033139

Miller, G. A., & Rockstroh, B. (2013). Endophenotypes in psychopathology research: where do we stand? *Annual Review of Clinical Psychology, 9*(1), 177-213. doi: doi:10.1146/annurev-clinpsy-050212-185540

Ministry of Health, Singapore (2014, Aug 1). *Admission for normal (vaginal) delivery*. Retrieved from: http://www.moh.gov.sg/content/moh_web/home/costs_and_financing/HospitalBillSize/delivery_normal.html

Mitchell, S., Brian, J., Zwaigenbaum, L., Roberts, W., Szatmari, P., Smith, I., & Bryson, S. (2006). Early language and communication development of infants later diagnosed with Autism Spectrum Disorder. *Journal of Developmental & Behavioral Pediatrics, 27*(2), S69-S78.

Mukherjee, R., Layton, M., Yacoub, E., & Turk, J. (2011). Autism and autistic traits in people exposed to heavy prenatal alcohol: data from a clinical series of 21 individuals and nested case control study. *Advances in Mental Health and Intellectual Disabilities, 5*(1), 42-49. doi: http://dx.doi.org/10.5042/amhid.2011.0015

Mullen, E. M. (1995). *Mullen scales of early learning*: AGS Circle Pines, MN.

Newschaffer, C. J., Croen, L. A., Daniels, J., Giarelli, E., Grether, J. K., Levy, S. E., . . . Windham, G. C. (2007). The epidemiology of Autism

Spectrum Disorders. *Annual Review of Public Health, 28*(1), 235-258. doi: doi:10.1146/annurev.publhealth.28.021406.144007

Osterling, J., & Dawson, G. (1994). Early recognition of children with autism: A study of first birthday home videotapes. *Journal of Autism and Developmental Disorders, 24*(3), 247-257. doi: 10.1007/bf02172225

Ozonoff, S., Iosif, A.-M., Baguio, F., Cook, I. C., Hill, M. M., Hutman, T., . . . Young, G. S. (2010). A prospective study of the emergence of early behavioral signs of Autism. *Journal of the American Academy of Child & Adolescent Psychiatry, 49*(3), 256-266.e252. doi: http://dx.doi.org/10.1016/j.jaac.2009.11.009

Ozonoff, S., Young, G. S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., . . . Stone, W. L. (2011). Recurrence risk for Autism Spectrum Disorders: a baby siblings Research Consortium study. *Pediatrics, 128*(3), e488-e495. doi: 10.1542/peds.2010-2825

Palomo, R., Belinchon, M., & Ozonoff, S. (2006). Autism and family home movies: a comprehensive review. *Journal of Developmental & Behavioral Pediatrics, 27*(2), S59-S68.

Piven, J., Palmer, P., Jacobi, D., Childress, D., & Arndt, S. (1997). Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. *American Journal of Psychiatry, 154*(2), 185-190.

Piven, J., Simon, J. O. N., Chase, G. A., Wzorek, M., Landa, R., Gayle, J., & Folstein, S. (1993). The etiology of autism: pre-, peri- and neonatal Factors. *Journal of the American Academy of Child and Adolescent Psychiatry, 32*(6), 1256-1263.

Plomin, R., Haworth, C. M. A., & Davis, O. S. P. (2009). Common disorders are quantitative traits. *Nature Reviews Genetics, 10*(12), 872-878. doi: 10.1038/nrg2670

Putnam, S. P., Gartstein, M. A., & Rothbart, M. K. (2006). Measurement of fine-grained aspects of toddler temperament: The Early Childhood Behavior Questionnaire. *Infant Behavior and Development, 29*(3), 386-401. doi: http://dx.doi.org/10.1016/j.infbeh.2006.01.004

Reichenberg, A., Gross, R., Weiser, M., Bresnahan, M., Silverman, J., Harlap, S., . . . Susser, E. (2006). Advancing paternal age and autism. *Archives of General Psychiatry, 63*(9), 1026-1032. doi: 10.1001/archpsyc.63.9.1026

Reiersen, A. M., Constantino, J. N., Grimmer, M., Martin, N. G., & Todd, R. D. (2008). Evidence for shared genetic influences on self-reported ADHD and autistic symptoms in young adult Australian twins. *Twin Research and Human Genetics, 11*(06), 579-585. doi: doi:10.1375/twin.11.6.579

Reynolds, S., & Lane, S. J. (2008). Diagnostic validity of sensory over-responsivity: A review of the literature and case reports. *J Autism Dev Disord, 38*(3), 516-529.

Rieffe, C., Oosterveld, P., Terwogt, M. M., Mootz, S., Van Leeuwen, E., & Stockmann, L. (2011). Emotion regulation and internalizing symptoms in children with autism spectrum disorders. *Autism, 15*(6), 655-670.

Ritvo, E. R., Freeman, B. J., Mason-Brothers, A., Mo, A., & Ritvo, A. M. (1985). Concordance for the syndrome of autism in 40 pairs of afflicted twins. *American Journal of Psychiatry, 142*, 74-77.

Robinson, E., Koenen, K., McCormick, M., Munir, K., Hallett, V., Happé, F., . . . Ronald, A. (2012). A multivariate twin study of autistic traits in 12-Year-Olds: Testing the Fractionable Autism Triad Hypothesis. *Behavior Genetics, 42*(2), 245-255. doi: 10.1007/s10519-011-9500-3

Robinson, E. B., Koenen, K. C., McCormick, M. C., & et al. (2011). Evidence that autistic traits show the same etiology in the general population and at the quantitative extremes (5%, 2.5%, and 1%). *Archives of General Psychiatry, 68*(11), 1113-1121. doi: 10.1001/archgenpsychiatry.2011.119

Rogers, S. J. (2009). What are infant siblings teaching us about autism in infancy? *Autism Research, 2*(3), 125-137. doi: 10.1002/aur.81

Rogers, S. J., & DiLalla, D. L. (1990). Age of symptom onset in young children with Pervasive Developmental Disorders. *Journal of the American Academy of Child & Adolescent Psychiatry, 29*(6), 863-872. doi: http://dx.doi.org/10.1097/00004583-199011000-00004

Rogers, S. J., Hepburn, S. L., Stackhouse, T., & Wehner, E. (2003). Imitation performance in toddlers with autism and those with other developmental disorders. *Journal of Child Psychology and Psychiatry, 44*(5), 763-781. doi: 10.1111/1469-7610.00162

Ronald, A., Happé, F., Bolton, P., Butcher, L. M., Price, T. S., Wheelwright, S., . . . Plomin, R. (2006a). Genetic Heterogeneity Between the Three Components of the Autism Spectrum: A Twin Study. *Journal of the American Academy of Child & Adolescent Psychiatry, 45*(6), 691-699. doi: http://dx.doi.org/10.1097/01.chi.0000215325.13058.9d

Ronald, A., Happé, F., Dworzynski, K., Bolton, P., & Plomin, R. (2010). Exploring the relation between prenatal and neonatal complications and later autistic-like features in a representative community sample of twins. *Child Development, 81*(1), 166-182. doi: 10.1111/j.1467-8624.2009.01387.x

Ronald, A., Happé, F., & Plomin, R. (2005). The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. *Developmental Science, 8*(5), 444-458. doi: 10.1111/j.1467-7687.2005.00433.x

Ronald, A., Happé, F., Price, T. S., Baron-Cohen, S., & Plomin, R. (2006b). Phenotypic and genetic overlap between autistic traits at the extremes of the general population. *Journal of the American Academy of Child and Adolescent Psychiatry, 45*(10), 1206-1214.

Ronald, A., & Hoekstra, R. (2014). Progress in understanding the causes of autism spectrum disorders and autistic traits: twin studies from 1977 to the present day. *Behavior Genetics of Psychopathology* (pp. 33-65). New York: Springer.

Ronald, A., & Hoekstra, R. A. (2011). Autism Spectrum Disorders and autistic traits: A decade of new twin studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 156*(3), 255-274. doi: 10.1002/ajmg.b.31159

Ronald, A., Larsson, H., Anckarsater, H., & Lichtenstein, P. (2011). A twin study of autism symptoms in Sweden. *Molecular Psychiatry, 16*(10), 1039-1047. doi: doi:10.1038/mp.2010.82

Rosenthal, A. N., & Paterson-Brown, S. (1998). Is there an incremental rise in the risk of obstetric intervention with increasing maternal age? *BJOG: An International Journal of Obstetrics & Gynaecology, 105*(10), 1064-1069. doi: 10.1111/j.1471-0528.1998.tb09937.x

Rothbart, M., & Bates, J. (1998). Temperament. In W. Damon & N. Eisenberg (Eds.), *Handbook of child psychology: Vol. 3, Social, emotional, and personality development.* (5th ed., pp. 105-176). NJ: Wiley.

Rothbart, M. K., Chew, K. H., & Gartstein, M. A. (2001). Assessment of temperament in early development. In L. Singer & P. S. Zeskind (Eds.), *Biobehavioral assessment of the infant* (pp. 190–208). New York: Guilford.

Rothbart, M., Derryberry, D., & Hershey, K. (2000). Stability of temperament in childhood: laboratory infant assessment to parent report at seven years. In V. Molfese & D. Molfese (Eds.), *Temperament and personality development across the lifespan* (pp. 37-86). Mahwah: Lawrence Erlbaum.

Rothbart, M. K., & Putnam, S. P. (2002). Temperament and socialization. In C. Nelson & M. Luciana (Eds.), *Paths to successful development: Personality in the life course* (pp. 19-45). Cambridge: Cambridge University Press.

Rutter, M. (1978). Diagnosis and definition of childhood autism. *Journal of autism and childhood schizophrenia, 8*(2), 139-161. doi: 10.1007/bf01537863

Rutter, M., & Schopler, E. (1987). Autism and pervasive developmental disorders: Concepts and diagnostic issues. *Journal of Autism and Developmental Disorders, 17*(2), 159-186. doi: 10.1007/bf01495054

Sandin, S., Hultman, C. M., Kolevzon, A., Gross, R., MacCabe, J. H., & Reichenberg, A. (2012). Advancing maternal age is associated with increasing risk for Autism: A review and meta-analysis. *Journal of the American Academy of Child & Adolescent Psychiatry, 51*(5), 477-486.e471. doi: http://dx.doi.org/10.1016/j.jaac.2012.02.018

Sandin, S., Kolevzon, A., Levine, S. Z., Hultman, C. M., & Reichenberg, A. (2013). Chapter 2.6 - Parental and perinatal risk factors for Autism: Epidemiological findings and potential mechanisms. In J. D. Buxbaum & P. R. Hof (Eds.), *The Neuroscience of Autism Spectrum Disorders* (pp. 195-202). San Diego: Academic Press.

Schroeder, J. H., Desrocher, M., Bebko, J. M., & Cappadocia, M. C. (2010). The neurobiology of autism: Theoretical applications. *Research in Autism Spectrum Disorders, 4*(4), 555-564.

Shea, A. K., & Steiner, M. (2008). Cigarette Smoking During Pregnancy. *Nicotine & Tobacco Research, 10*(2), 267-278. doi: 10.1080/14622200701825908

Shuster, J., Perry, A., Bebko, J., & Toplak, M. (2014). Review of factor analytic studies examining symptoms of Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders, 44*(1), 90-110. doi: 10.1007/s10803-013-1854-3

Sigman, M. D., Kasari, C., Kwon, J.-H., & Yirmiya, N. (1992). Responses to the negative emotions of others by autistic, mentally retarded, and

normal children. *Child Development*, *63*(4), 796-807. doi: 10.1111/j.1467-8624.1992.tb01662.x

Soh, S.-E., Tint, M. T., Gluckman, P. D., Godfrey, K. M., Rifkin-Graboi, A., Chan, Y. H., . . . Group, t. G. S. (2013). Cohort Profile: Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort study. *International Journal of Epidemiology*. doi: 10.1093/ije/dyt125

Soto, S., Linas, K., Jacobstein, D., Biel, M., Migdal, T., & Anthony, B. J. (2014). A review of cultural adaptations of screening tools for autism spectrum disorders. *Autism*. doi: 10.1177/1362361314541012

Sparrow, S. S., Balla, D. A., & Cicchetti, D. V. (1984). *Vineland adaptive behavior scales: Interview edition, survey form manual*: American Guidance Service Circle Pines, MN.

Spence, S. J. (2004). *The genetics of autism.* Paper presented at the Seminars in Pediatric Neurology.

Stein, D., Weizman, A., Ring, A., & Barak, Y. (2006). Obstetric complications in individuals diagnosed with autism and in healthy controls. *Comprehensive Psychiatry, 47*(1), 69-75. doi: http://dx.doi.org/10.1016/j.comppsych.2005.01.001

Stevens, S. A., Nash, K., Koren, G., & Rovet, J. (2012). Autism characteristics in children with fetal alcohol spectrum disorders. *Child Neuropsychology, 19*(6), 579-587. doi: 10.1080/09297049.2012.727791

Stilp, R. L. H., Gernsbacher, M. A., Schweigert, E. K., Arneson, C. L., & Goldsmith, H. H. (2010). Genetic variance for Autism screening items

in an unselected sample of toddler-age twins. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*(3), 267-276.

Sullivan, M., Finelli, J., Marvin, A., Garrett-Mayer, E., Bauman, M., & Landa, R. (2007). Response to joint attention in toddlers at risk for Autism Spectrum Disorder: a prospective study. *Journal of Autism and Developmental Disorders, 37*(1), 37-48. doi: 10.1007/s10803-006-0335-3

Sun, X., & Allison, C. (2010). A review of the prevalence of Autism Spectrum Disorder in Asia. *Research in Autism Spectrum Disorders, 4*(2), 156-167. doi: http://dx.doi.org/10.1016/j.rasd.2009.10.003

Swinkels, S. N., Dietz, C., van Daalen, E., Kerkhof, I. G. M., van Engeland, H., & Buitelaar, J. (2006). Screening for Autistic spectrum in children aged 14 to 15 months. I: The development of the Early Screening of Autistic Traits Questionnaire (ESAT). *Journal of Autism and Developmental Disorders, 36*(6), 723-732. doi: 10.1007/s10803-006-0115-0

Tan, S.H. (2010). Multilingual Infant Vocabulary in Singapore. In M. Cruz-Ferreira (Ed.), *Multilingual Norms* (pp. 113-140). Germany: Peter Lang.

Thal, D., & Bates, E. (1988). Language and gesture in late talkers. *Journal of Speech, Language, and Hearing Research, 31*(1), 115-123. doi: 10.1044/jshr.3101.115

Thomas, A., & Chess, S. (1977). *Temperament and development*. New York: Brunner/Mazel.

Thomas, A., Chess, S., & Birch, H. G. (1968). *Temperament and behavior disorders in children*. New York: New York University Press.

Thomas, A., Chess, S., Birch, H. G., Hertzig, M. E., & Korn, S. (1963). *Behavioral individuality in early childhood*. New York, NY, US: New York University Press.

Tordjman, S., Coulon, N., Kermarrec, S., Cohen, D., Bronsard, G., Weismann-Arcache, C., ... & Xavier, J. (2014). Gene X environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Frontiers in Psychiatry, 53*(5), 1-17.

Tsai, L., & Stewart, M. (1983). Etiological implication of maternal age and birth order in infantile autism. *Journal of Autism and Developmental Disorders, 13*(1), 57-65. doi: 10.1007/bf01531359

Turner, T., Pihur, V., & Chakravarti, A. (2011). Quantifying and modeling birth order effects in autism. *PloS one, 6*(10), e26418. doi: 10.1371/journal.pone.0026418

Virkud, Y. V., Todd, R. D., Abbacchi, A. M., Zhang, Y., & Constantino, J. N. (2009). Familial aggregation of quantitative autistic traits in multiplex versus simplex autism. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 150B*(3), 328-334. doi: 10.1002/ajmg.b.30810

Wakabayashi, A., Baron-Cohen, S., Uchiyama, T., Yoshida, Y., Tojo, Y., Kuroda, M., & Wheelwright, S. (2007). The Autism-Spectrum Quotient (AQ) Children's Version in Japan: a cross-cultural comparison. *Journal of Autism and Developmental Disorders, 37*, 491 - 500.

Walker-Andrews, A. S. (1998). Emotions and social development: Infants' recognition of emotions in others. *Pediatrics, 102*(Supplement E1), 1268-1271.

Werner, E., & Dawson, G. (2005). Validation of the phenomenon of autistic regression using home videotapes. *Archives of General Psychiatry, 62*(8), 889-895. doi: 10.1001/archpsyc.62.8.889

Werner, E., Dawson, G., Osterling, J., & Dinno, N. (2000). 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, 30*(2), 157-162. doi: 10.1023/a:1005463707029

Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., . . . Wakabayashi, A. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemizing Quotient-Revised (SQ-R) and Empathy Quotient (EQ). *Brain Research, 1079*(1), 47-56. doi: http://dx.doi.org/10.1016/j.brainres.2006.01.012

Wilcox, A. J. (2001). On the importance—and the unimportance— of birthweight. *International Journal of Epidemiology, 30*(6), 1233-1241. doi: 10.1093/ije/30.6.1233

Williams, J., Allison, C., Scott, F., Bolton, P., Baron-Cohen, S., Matthews, F., & Brayne, C. (2008). The Childhood Autism Spectrum Test (CAST): sex differences. *Journal of Autism and Developmental Disorders, 38*, 1731 - 1739.

Williams, K., Helmer, M., Duncan, G. W., Peat, J. K., & Mellis, C. M. (2008). Perinatal and maternal risk factors for autism spectrum disorders in

New South Wales, Australia. *Child: Care, Health and Development, 34*(2), 249-256. doi: 10.1111/j.1365-2214.2007.00796.x

Wong, H. S., Huertas-Ceballos, A., Cowan, F. M., & Modi, N. (2014). Evaluation of early childhood social-communication difficulties in children born preterm using the Quantitative Checklist for Autism in Toddlers. *The Journal of Pediatrics, 164*(1), 26-33.e21. doi: http://dx.doi.org/10.1016/j.jpeds.2013.07.013

Yeates, K. O., Bigler, E. D., Dennis, M., Gerhardt, C. A., Rubin, K. H., Stancin, T., . . . Vannatta, K. (2007). Social outcomes in childhood brain disorder: a heuristic integration of social neuroscience and developmental psychology. *Psychological bulletin, 133*(3), 535. doi: 10.1037/0033-2909.133.3.535

Yirmiya, N., & Charman, T. (2010). The prodrome of autism: early behavioral and biological signs, regression, peri- and post-natal development and genetics. *Journal of Child Psychology and Psychiatry, 51*(4), 432-458. doi: 10.1111/j.1469-7610.2010.02214.x

Zwaigenbaum, L., Bryson, S., Lord, C., Rogers, S., Carter, A., Carver, L., . . . Yirmiya, N. (2009). Clinical assessment and management of toddlers with suspected Autism Spectrum Disorder: insights from studies of high-risk infants. *Pediatrics, 123*(5), 1383-1391. doi: 10.1542/peds.2008-1606

Zwaigenbaum, L., Szatmari, P., Jones, M. B., Bryson, S. E., Maclean, J. E., Mahoney, W. J., . . . Tuff, L. (2002). Pregnancy and birth complications in Autism and liability to the Broader Autism Phenotype. *Journal of the American Academy of Child & Adolescent*

Psychiatry, 41(5), 572-579. doi: http://dx.doi.org/10.1097/00004583-200205000-00015

Appendix A.

Quantitative CHecklist of Autism in Toddlers (Q-CHAT)

Please answer the following questions about your child by ticking the appropriate circle.
Try to answer EVERY question if you can.

1. Does your child look at you when you call his/her name?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

2. How easy is it for you to get eye contact with your child?

- ○ very easy
- ○ quite easy
- ○ quite difficult
- ○ very difficult
- ○ impossible

3. When your child is playing alone, does s/he line objects up?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

4. Can other people easily understand your child's speech?

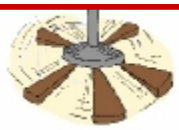
- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never
- ○ my child does not speak

5. Does your child point to indicate that s/he wants something (e.g. a toy that is out of reach)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

6. Does your child point to share interest with you (e.g. pointing at an interesting sight)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never



7. How long can your child's interest be maintained by a spinning object (e.g. washing machine, electric fan, toy car wheels)?

- ○ several hours
- ○ half an hour
- ○ ten minutes
- ○ a couple of minutes
- ○ less than a minute

8. How many words can your child say?

- ○ none—s/he has not started speaking yet
- ○ less than 10 words
- ○ 10–50 words
- ○ 51–100 words
- ○ over 100 words

9. Does your child pretend (e.g. care for dolls, talk on a toy phone)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

10. Does your child follow where you're looking?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

11. How often does your child sniff or lick unusual objects?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

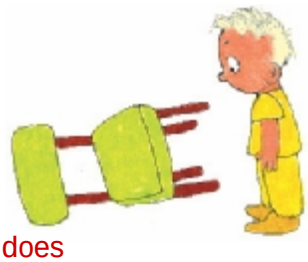
12. Does your child place your hand on an object when s/he wants you to use it (e.g. on a door handle when s/he wants you to open the door, on a toy when s/he wants you to activate it)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

13. Does your child walk on tiptoe?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

14. How easy is it for your child to adapt when his/her routine changes or when things are out of their usual place?



- ○ very easy
- ○ quite easy
- ○ quite difficult
- ○ very difficult
- ○ impossible

15. If you or someone else in the family is visibly upset, does your child show signs of wanting to comfort them (e.g. stroking their hair, hugging them)?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

16. Does your child do the same thing over and over again (e.g. running the tap, turning the light switch on and off, opening and closing doors)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

17. Would you describe your child's first words as:

- ○ very typical
- ○ quite typical
- ○ slightly unusual
- ○ very unusual
- ○ my child doesn't speak

18. Does your child echo things s/he hears (e.g. things that you say, lines from songs or movies, sounds)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

19. Does your child use simple gestures (e.g. wave goodbye)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

20. Does your child make unusual finger movements near his/her eyes?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

21. Does your child spontaneously look at your face to check your reaction when faced with something unfamiliar?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

22. How long can your child's interest be maintained by just one or two objects?

- ○ most of the day
- ○ several hours
- ○ half an hour
- ○ ten minutes
- ○ a couple of minutes

23. Does your child twiddle objects repetitively (e.g. pieces of string)?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

24. Does your child seem oversensitive to noise?

- ○ always
- ○ usually
- ○ sometimes
- ○ rarely
- ○ never

25. Does your child stare at nothing with no apparent purpose?

- ○ many times a day
- ○ a few times a day
- ○ a few times a week
- ○ less than once a week
- ○ never

Appendix B.

SingHealth Centralized Institutional Review Board Approval Letter & Renewal of NHG Domain Specific Review Board Appeal

SingHealth
Defining Tomorrow's Medicine

Tel: (65) 6225 0488
Fax: (65) 6557 2138
Singapore Health Services Pte Ltd
31 Third Hospital Avenue
#03-03 Bowyer Block C
Singapore 168753
www.singhealth.com.sg
UEN No. 200002698Z

CIRB Ref: **2009/280/D**

07 March 2014

A/Prof Fabian Yap
Department of Endocrinology
KK Women's and Children's Hospital

Dear A/Prof Yap

SINGHEALTH CENTRALISED INSTITUTIONAL REVIEW BOARD (CIRB) RE-APPROVAL

Study Title: GUSTO - Growing Up in Singapore Towards Healthy Outcomes

We are pleased to inform you that the SingHealth Centralised Institutional Review Board D has re-approved the above research project to be conducted in KK Women's and Children's Hospital. The approval period is from **07 March 2014 to 06 March 2015**.

The documents reviewed are:

a) Study Status Report dated 24 January 2014

The SingHealth Centralised IRB operates in accordance with the ICH/ Singapore Guideline for Good Clinical Practices, and with the applicable regulatory requirement(s).

Please note that annual IRB renewal is required and the review is based on the Study Status Report submitted. It is the Principal Investigator's responsibility to submit a Study Status Report for the study at least one month before the expiry date of the study for renewal of IRB approval. No study should continue beyond the expiry date unless the IRB has reviewed the Study Status Report and approved the continuation of the study. Failure to submit the Study Status Report can result in study suspension.

Yours sincerely,

Dr Steve Yang
Chairman
SingHealth Centralised Institutional Review Board D

Cc: Institution Representative, KKH
Chairman, Division of Medicine, KKH

3 Fusionopolis Link
#03-08 Nexus@one-north
Singapore 138543

Tel: 6496 6600 Fax: 6486 6870

www.nhg.com.sg
RCB No. 200002150H

NHG DSRB Ref: 2009/00021

02 January 2014

A/Prof Lee Yung Seng
Department of Paediatrics
National University Hospital

Dear A/Prof Lee

RENEWAL OF NHG DOMAIN SPECIFIC REVIEW BOARD (DSRB) APPROVAL

STUDY TITLE: GUSTO - Growing Up in Singapore Towards healthy Outcomes

Sub study – Substudy in women conceived through assisted reproductive technology

Sub study – Studying body composition in Neonates using MRI

Sub study- Birth Parameters, early life course factors and association with myopia in young children [Gusto-Eye]

Sub study – Study of Maternal Microbiota and its Impact on Child's Development

We are pleased to inform you that the NHG DSRB has renewed the approval for the application as titled above, being conducted in **National University Hospital**. The approval period is from **02 January 2014** to **01 January 2015**.

The documents reviewed are:

1) NHG DSRB Study Status Report Form ID: **2009/00021-SRF0005**
2) NHG DSRB Application Form: **Version No. 17**
3) Study Protocol: Version 1.0 dated 4/12/2008
4) Main GUSTO- Participant Information Sheet and Consent Form (NUHS): Version 1.12 dated 06/09/2011
5) Healthcare Services Expenditure Module (Month 15 Visit): Version 1.0 dated 21/02/2011
6) Participant Information Sheet and Consent Form (Spouse): Version 1.5 dated 1/12/2011 (NUHS)
7) Main GUSTO- Participant Information Sheet and Consent Form (NUHS) – Addendum: Version 1.1 dated 18/04/2011
8) Neurocognitive Function in Six Month Olds – Participant Information Sheet and Consent Form: Version 1.0 dated 19/05/2010 (NUHS)
9) Neonatal Neurocognitive Function – Participant Information Sheet and Consent Form: Version 1.0 dated 19/05/2010 (NUHS)
10) Addendum – Participant Information Sheet and Consent Form: Version dated 29/04/2010 (NUHS)
11) Neurocognitive Function in Children - Participant Information Sheet and Consent Form (NUHS): Version 1.5 dated 27/11/2013
12) Patient Information Sheet Addendum: Version 1.0 dated 18/05/2012

13) GUSTO 1st Clinic Visit Questionnaires: Version dated 31/03/2009
14) GUSTO Eligibility Questionnaires: Version dated 31/03/2009
15) GUSTO Newsletter Jun-Sep 2012 Issue
16) GUSTO Newsletter: Jan-May 2013 Issue 4
17) Peabody vocabulary test scoring sheet

18) Peabody vocabulary test pictures
19) 24 Months Neurocognition Visit Brochure : Version dated 07/02/2013
20) 3 year old Neurocognition Visit Brochure : Version dated 04/12/2012
21) School Readiness Test- Participant Information Sheet and Consent Form: Version 1.0 dated 27/11/2013
22) Gusto 4 to 9 years follow up- Participant Information Sheet and Consent Form: Version 1.0 dated 22/10/2013
23) School Readiness Test Brochure: Version 1.0 dated 29/08/2013
24) Gusto 4 to 9 years follow up Brochure : Version 1.0 dated 09/09/2013
25) Life Experiences Survey: Version 1.0 dated 29/08/2013
26) Child Behavior Checklist : Version 1.0 dated 29/08/2013
27) 48 Months School Readiness Test Protocol : Version 1.0 dated 24/10/2013
28) 36 Months Brochure
29) GUSTO Brochure
30) GUSTO A3 Poster
31) GUSTO-Eligibility Questionnaire: final, dated 22 October 2009
32) GUSTO-1st Clinic Visit Questionnaire: dated 12 November 2009
33) Birth Cohort – 26 Weeks Questionnaire: dated 22 December 2009
34) Birth Cohort – Mother's CRF 26 week: dated 07 June 2010
35) CRF Antenatal Scans: dated 21 August 2009
36) GUSTO CRF Term Baby: dated 20 August 2010
37) Annex 1 CRF – Hypertension: dated 20 August 2010
38) Annex 2 CRF – Pre-eclampsia: dated 25 November 2009
39) Annex 3 CRF – Gestational diabetes: dated 20 August 2010
40) Annex 4 CRF – IUGR: dated 20 August 2010
41) Annex 5 CRF – Mutiple Pregnancy: dated 18 January 2010
42) Annex 6 GUSTO CRF – NICU baby: dated 25 October 2010
43) NICU Feeding Log: dated 25 October 2010
44) MRI CRF – RVS: dated 28 February 2010
45) MRI CRF – Appendix: dated 16 July 2010
46) MRI Record Form – NUH: dated 07 June 2010
47) 3 Week Infancy Questionnaires: dated 18 August 2010
48) Week 3 Infancy CRF: dated 30 August 2010
49) Month 3 Infancy Questionnaires: dated 18 August 2010
50) Month 3 Infancy CRF: dated 30 August 2010
51) Month 6 Infancy Questionnaires: dated 18 August 2010
52) Month 6 Infancy CRF: dated 30 August 2010
53) Month 6 Infancy EYE CRF: dated 18 August 2010
54) Month 6 Environment Questionnaire: dated 15 May 2010
55) Month 9 Infancy Questionnaires: dated 21 August 2010
56) Month 9 Infancy CRF: dated 30 August 2010
57) Month 15 Infancy CRF : Version dated 21/02/2011
58) Month 15 Infancy Questionnaire : Version No. 1.2 dated 06/08/2011
59) Month 18 Infancy CRF: Version dated 28/07/2011
60) Month 18 Infancy Questionnaire : Version 1.4 dated 06/08/2011
61) Month 24 Infancy CRF : Version dated 11/11/2011
62) Month 24 Child Questionnaire : Version No. 1.3 dated 30/01/2012
63) Month 36 CRF : Version dated 04/12/2012
64) Month 36 Child Questionnaire : Version dated 25/02/2013
65) GUSTO Mother Food Diary: Final
66) Infant Feeding Diary
67) My First Food Diary 100624, photo-frame: Final
68) 3 Day Food Diary – Infants & Children: dated 14 October 2010
69) Sleep-Wake Diary – Mom: Version 3
70) Sleep-Wake Diary – Child: Version 3
71) Brief Screening Questionnaire for Infant Sleep (BISQ) :Final Version
72) MGI Shen STATIAD6936
73) Lydon Maternal Health and Well Being: Version dated 28/02/2010
74) Edinburgh Postnatal Depression Scale (EPDS)
75) STAI (60 months)
76) BDI-II
77) Questionnaires on DH: dated 18 September 2009 RVS
78) Pittsburgh Sleep Quality Index – Final Version: dated 05 March 2010
79) GUSTO BEBQ – Month 3: dated 16 June 2010
80) Carey Temperament Scales 1-4 M: Final Version
81) Developmental Milestone: Final Version
82) Birth Cohort LBQ : Final Version

83) 9 Months Questionnaire – Compiled: Final Version
84) Carey Temperament Scales 4-11 month
85) Brief Screening Questionnaire for Infant Sleep (BISQ)
86) Parents Evaluation of Developmental Status (PEDS)
87) Parents Evaluation of Developmental Status Milestones – PEDS-DM
88) ASQ-3 Ages & Stages Questionnaires
89) Ages & Stages Questionnaires: Social Emotional
90) My GUSTO Study Diary Health Record: dated 25 August 2010
91) GUSTO Hubble Questionnaire: dated 12 August 2010
92) GUSTO Hubble Study Diary – CRF: dated 12 August 2010
93) Month 3 Infancy Questionnaires: Version 1.1 dated 28/02/2010
94) Week 26-28 Clinic Visit Interviewer-administered Questionnaire (Mother): Version dated 22/12/2009
95) Recruitment Visit 1st Clinic Questionnaire: Version dated 12/11/2009
96) Recruitment Visit Eligibility Questionnaire: Version dated 22/10/2009
97) Month 12 Infancy Questionnaires: Version 1.1 dated 03/12/2010
98) Month 12 Infancy CRF: Version dated 28/12/2010
99) 12 Months Combined Questionnaire – ITSEA Parent Form: Version dated 2/12/2010
100) Childhood Literacy – Parents' Questionnaire
101) Developmental Milestones of Early Literacy – 6 to 12 months
102) Singapore English Communicative Development Inventory – Words and Gestures (Abbreviated version)
103) LYDON Health and Well Being of Mothers and their Newborns – 12 months
104) Month 12 Environment Questionnaire : Version 1.0 dated 19/11/2010
105) Child Eating Behaviour Questionnaire (CEBQ) – 12 months
106) Newsletter (August – October 2011 Issue 1)

Substudy- Birth Parameters, early life course factors and association with myopia in young children [Gusto-Eye]

1) Participant Information Sheet and Consent Form: Version No. 1.1 dated 26/08/2013
2) Gusto 36 Months Eye Measurements: Version No. 1.0 dated 03/09/2013

Substudy in women conceived through assisted reproductive technology

1) Sub-study : Studying in Women Conceived Through Assisted Reproductive Technology: Version 1.4 dated 2/09/2011 (NUH)
2) Sub-study : Studying in Women Conceived Through Assisted Reproductive Technology (Spouse): Version 1.3 dated 1/12/2011 (NUHS)

Studying body composition in Neonates using MRI

1) Study Protocol: Version 1.0
2) Participant Information Sheet and Consent Form: Version 1.2 dated 19/05/2010 (NUHS)
3) Addendum – 1-2 month Visit Body Composition Follow Up: Version 1.0 dated 18/01/2011 (NUH)
4) Sub-study : Studying Body Composition in Neonates Follow Up Visit – 1-2mth and 6 month Visit Body Composition Follow Up Addendum: Version 1.1 dated 9/06/2011 (NUH)

Study of Maternal Microbiota and its Impact on Child's Development

1) Participant Information Sheet and Consent Form: Version 1.0 dated 19/01/2011

The documents acknowledged are:

2) GUSTO websites
3) GUSTO short video on Magnetic Resonance Imaging
4) NUH Informed Consent Form Chinese Version:
a. Informed Consent Form: Version 1.6 24 July 2009
b. Informed Consent Form: Version 1.8 20 October 2009
c. Informed Consent Form: Version 1.10 28 June 2010
d. GUSTO spouse consent: Version 1.0 31 March 2009
e. GUSTO Spouse consent: Version 1.1 21 August 2009
f. GUSTO Spouse consent: Version 1.4 28 June 2010
g. Day 3 MRI consent form: V1.0 15 October 2009
h. Day 3 MRI consent form: V1 2 November 2009
i. Day 3 MRI consent form: V1.2 19 May 2010
j. Addendum BM & BIA: V1.0 19 May 2010

k. Neurocogition EEG Consent: V1.0 19 May 2010
l. Neurocognition 6mth Consent: V1.0 19 May 2010
5) NUH Informed Consent Form Malay Version:
a. Informed Consent Form: Version 1.6 24 July 2009
b. Informed Consent Form: Version 1.8 20 October 2009
c. GUSTO spouse consent: Version 1.0 31 March 2009
d. MRI consent form: V1.1 2 November 2009
e. Addendum BM & BIA: V1.0 19 May 2010
f. Neurocogition EEG Consent: V1.0 19 May 2010
g. Neurocognition 6mth Consent: V1.0 23 June 2010
6) NUH Informed Consent Form Tamil Version:
a. Informed Consent Form: Version 1.6 24 July 2009
b. Informed Consent Form: Version 1.1 18 October 2009
c. MRI consent form: V1.1 2 November 2009
d. Addendum BM & BIA: V1.0 19 May 2010
e. Neurocogition EEG Consent: V1.0 19 May 2010
f. Neurocognition 6mth Consent
7) Translated Chinese Version Questionnaire 3 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI): V1 5 March 2010
b. BDI-II
c. BISQ
d. BEBQ
e. STAIB-AD
f. Carey Temperament Scales
g. Edinburgh Postnatal Depression Scale (EPDS)
h. Infancy Questionnaires: Version 1.1 dated 28/02/2010
i. Questionnaires on DH: Version dated 18/02/2010
8) Translated Malay Version Questionnaire 3 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI): V1 5 March 2010
b. BDI-II
c. BISQ
d. BEBQ
e. STAIB-AD
f. STAI-Form Y-1
g. STAI-Form Y-2
h. Carey Temperament Scales
i. Edinburgh Postnatal Depression Scale (EPDS)
j. Infancy Questionnaires: Version 1.1 dated 28/02/2010
k. Questionnaires on DH: Version dated 18/02/2010
9) Translated Tamil Version Questionnaire 3 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI)
b. BDI-II
c. BISQ
d. BEBQ
e. STAI-60
f. Carey Temperament Scales
g. Edinburgh Postnatal Depression Scale (EPDS)
h. Infancy Questionnaires: Version 1.1 dated 28/02/2010
i. Questionnaires on DH: Version dated 18/02/2010
10) Translated Chinese Version Questionnaire 3 Weeks Home Visit:
a. Infancy Questionnaires: Version 1.2 dated 15/12/2009
11) Translated Malay Version Questionnaire 3 Weeks Home Visit:
a. Infancy Questionnaires: Version 1.2 dated 15/12/2009
12) Translated Tamil Version Questionnaire 3 Weeks Home Visit:
a. Infancy Questionnaires: Version 1.2 dated 15/12/2009
13) Translated Chinese Version Questionnaire 6 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI): V1 5 March 2010
b. BISQ
c. Infancy Questionnaires: Version 1.1 dated 27/05/2010
d. Developmental Milestones of Early Literacy – 6 to 12 months
e. Environment Questionnaire: Version 1.0 dated 2010
f. Sleep Wake Diary - Child: Version 3 dated 6/04/2010
g. Sleep Wake Diary - Mom: Version 3 dated 6/04/2010
14) Translated Malay Version Questionnaire 6 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI): V1 15 March 2010
b. BISQ

c. Infancy Questionnaires: Version 1.1 dated 27/05/2010
d. Developmental Milestones of Early Literacy – 6 to 12 months
e. Environment Questionnaire: Version 1.0 dated 15/05/2010
15) Translated Tamil Version Questionnaire 6 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI)
b. BISQ
c. Infancy Questionnaires: Version 1.1 dated 27/05/2010
d. Developmental Milestones of Early Literacy – 6 to 12 months
e. Environment Questionnaire: Version 1.0 dated 15/05/2010
16) Translated Chinese Version Questionnaire 9 Months Home Visit:
a. Revised Infant Temperament Questionnaire
b. Infancy Questionnaires: Version 1.0 dated 21/08/2010
17) Translated Malay Version Questionnaire 9 Months Home Visit:
a. Revised Infant Temperament Questionnaire
b. Infancy Questionnaires: Version 1.0 dated 21/08/2010
18) Translated Tamil Version Questionnaire 9 Months Home Visit:
a. Ages & Stages Questionnaires
b. ASQ-SE
c. Carey Temperament Scales
d. New Carey Temperament Scales
e. PEDS Response Form
f. PEDS-DM
19) Translated Chinese Version Questionnaire 12 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI) : Version 1 dated 5/03/2010
b. BDI-II
c. Infancy Questionnaires: Version 1.1 dated 3/12/2010
d. Developmental Milestones of Early Literacy – 6 to 12 months
20) Translated Malay Version Questionnaire 12 Months Home Visit:
a. Pittsburgh Sleep Quality Index (PSQI) : Version 1 dated 15/03/2010
b. BDI-II
c. BISQ
d. Developmental Milestones of Early Literacy – 6 to 12 months
e. ITSEA Parent Form
f. LYDON Health and Well Being of Mothers and their Newborns – 12 months: Version dated 31/10/2010
g. Environment Questionnaire: Version 1.0 dated 19/11/2010
h. Infancy Questionnaires: Version 1.1 dated 3/12/2010
i. NUSCDI Infant: Version 1 shortened
21) Translated Tamil Version Questionnaire 12 Months Home Visit:
a. BDI-II
b. LYDON Health and Well Being of Mothers and their Newborns – 12 months: Version dated 31/10/2010
c. Environment Questionnaire: Version 1.0 dated 19/11/2010
d. Infancy Questionnaires: Version 1.1 dated 3/12/2010
e. Child Eating Behaviour Questionnaire (CEBQ) – 12 months: Version dated 19/11/2010
22) Translated Chinese Version Questionnaire Delivery Home Visit
a. Study Diary Health Record: Version dated 20/08/2010
23) Translated Malay Version Questionnaire Clinic:
a. 26 Weeks Questions: Version dated 22/12/2009
b. Birth Cohort 26 Weeks Questionnaire: Version dated 20/08/2009
c. Birth Cohort 26 Weeks Eligibility Questionnaire: Version 1 dated 16/08/2009
d. BDI-II
e. Edinburgh Postnatal Depression Scale (EPDS)
f. 1st Clinic Visit Questionnaire: Version dated 21/08/2010
g. Domestic Helper 26 Weeks: Version 1
h. LYDON Maternal Well Being of Mothers and their Newborns: Version dated 28/02/2010
i. STAI-Form Y-1
j. STAI-Form Y-2
k. Pittsburgh Sleep Quality Index (PSQI) : Version 1 dated 15/03/2010
24) Translated Tamil Version Questionnaire Clinic:
a. 26 Weeks Questions: Version dated 20/08/2009
b. Birth Cohort 26 Weeks Questionnaire: Version dated 22/12/2009
c. Birth Cohort 26 Weeks Eligibility Questionnaire: Version dated 9/07/2009
d. BDI-II
e. Edinburgh Postnatal Depression Scale (EPDS)
f. 1st Clinic Visit Questionnaire: Version dated 12/08/2010
g. Domestic Helper 26 Weeks: Version 1
h. LYDON Maternal Well Being of Mothers and their Newborns: Version dated 28/02/2010

i. STAI-60
j. Pittsburgh Sleep Quality Index (PSQI)
25) Translated Chinese Version Questionnaire Clinic:
a. Birth Cohort 26 Weeks Questionnaire: Version dated 3/08/2009
b. Birth Cohort 26 Weeks Questionnaire: Version dated 22/12/2009
c. Birth Cohort 26 Weeks Eligibility Questionnaire: Version 1 dated 16/08/2009
d. Birth Cohort 26 Weeks Eligibility Questionnaire: Version dated 22/10/2009
e. BDI-II
f. Edinburgh Postnatal Depression Scale (EPDS)
g. 1st Clinic Visit Questionnaire: Version dated 3/08/2009
h. 1st Clinic Visit Questionnaire: Version dated 12/11/2009
i. Domestic Helper 26 Weeks: Version 1
j. LYDON Maternal Well Being of Mothers and their Newborns: Version dated 28/02/2010
k. STAI-Form Y-1
l. Pittsburgh Sleep Quality Index (PSQI) : Version 1 dated 5/03/2010
m. DevOS Food Diary: Version dated 1/09/2010

Continued approval is conditional upon your compliance with the following requirements:

1. Only the approved Informed Consent Form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject should be given a copy of the signed consent form.

2. No deviation from, or changes of the protocol should be implemented without documented approval from the NHG DSRB, except where necessary to eliminate apparent immediate hazard(s) to the study subjects.

3. Any deviation from, or a change of, the protocol to eliminate an immediate hazard should be promptly reported to the NHG DSRB within seven calendar days.

4. Please note that for studies requiring Clinical Trial Certificate, apart from the approval from NHG DSRB, no deviation from, or changes of the Research Protocol and Informed Consent Form should be implemented without documented approval from the Health Sciences Authority unless otherwise advised by the Health Sciences Authority.

5. Please submit the following to the NHG DSRB:

a. All Unanticipated Problems Involving Risk To Subjects Or Others (UPIRTSOs) must be reported to the NHG DSRB. All problems involving local deaths must be reported immediately within 24 hours after first knowledge by the Investigator, regardless of the casuality and expectedness of the death. All other problems must be reported as soon as possible but not later than seven calendar days after first knowledge by the Investigator.

b. Report(s) on any new information that may adversely affect the safety of the subject or the conduct of the study.

c. NHG DSRB Study Status Report Form – this is to be submitted 4 to 6 weeks prior to expiry of the approval period. The study cannot continue beyond **01 January 2015** until approval is renewed by the NHG DSRB.

d. Study completion – this is to be submitted using the NHG DSRB Study Status Report Form within 4 to 6 weeks of study completion or termination.

6. Established since May 2006, the NHG Research Quality Management (RQM) Program seeks to promote the responsible conduct of research in a research culture with high ethical standards, identify potential systemic weaknesses and make recommendations for continual improvement. Hence, this research study may be randomly selected for a review by the Research Quality Management (RQM) team. For more information, please visit www.research.nhg.com.sg.

Yours Sincerely

A/Prof Low Yin Peng
Chairman
NHG Domain Specific Review Board D

Cc: Institutional Representative, NUH
c/o Research Office, NUH
Departmental Representative of Paediatrics, NUH

(This is an electronic-generated letter. No signature is required.)

Appendix C.

Normality statistics for key study variables

	Skewness (SE)	Standardized Skewness Coefficient	Kurtosis (SE)	Standardized Kurtosis Coefficient	Shapiro-Wilks Statistic (*p*)
Pregnancy/birth-related factors					
Overall suboptimality (N = 1089)	.85(.07)	11.5	.66(.15)	4.44	**.858 (.000)**
Temperament (3 months; N = 645)					
Activity	-.02 (.10)	-0.25	.26 (.19)	1.37	**.993 (.004)**
Rhythmicity	-.37 (.10)	-3.88	.20 (.19)	1.06	**.987 (.000)**
Approach	.10 (.10)	0.98	-.30 (.19)	-1.58	**.993 (.003)**
Adaptability	-.07 (.10)	-0.74	-.27 (.19)	-1.40	.996 (.087)
Intensity	-.12 (.10)	-1.24	.01 (.19)	0.50	**.995 (.035)**
Mood	-.09 (.10)	-0.92	.19 (.19)	0.99	**.995 (.004)**
Persistence	.34 (.10)	3.50	.10 (.19)	0.49	**.988 (.000)**
Distraction	.13 (.10)	1.39	-.39 (.19)	-2.01	**.991 (.001)**
Threshold	-.30 (.10)	-3.13	.55 (.19)	2.87	**.991 (.000)**
SECDI-Words and Gestures (12 months; N = 478)					
Gestures total score	-.21(.11)	-1.90	-.26(.22)	1.18	**.973(.000)**
ITSEA Competence Subscales (12 months; N = 493)					
Imitation/Play	-.02(.11)	0.18	-.29(.22)	1.32	**.984 (.000)**
Empathy	.47 (.11)	4.27	-.48 (.22)	2.18	**.952 (.000)**
Q-CHAT scores (18 months; N = 368)					
Total	-.06 (.13)	-0.46	.52 (.25)	2.05	.993 (.077)
Social factor	.88 (.13)	6.77	1.15(.25)	4.51	**.952 (.000)**
Non-social factor	.11(.13)	0.88	.17(.25)	0.67	.992 (.055)