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Investigating associations between birth order and autism diagnostic phenotypes

Running head: Birth order and autism

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Background: Birth order effects have been linked to variability in intelligence, educational attainment, and sexual orientation. First- and later-born children have been linked to an increased likelihood of an Autism Spectrum Disorder (ASD) diagnosis, with a smaller body of evidence implicating decreases in cognitive functioning with increased birth order. The present study investigated the potential association between birth order and ASD diagnostic phenotypes in a large and representative population sample. **Methods:** Data were obtained from an ongoing prospective diagnostic registry, collected between 1999 and 2017, including children (1-18 years of age, n = 5404) diagnosed with ASD in the state of Western Australia. Children with ASD were ranked relative to sibling's birth to establish birth order within families at time of ASD diagnosis. Information reported to the registry by health professionals at the time of diagnostic evaluation included demographic and family characteristics, functional abilities and intellectual capacity. **Results:** Adaptive functioning and intelligence scores decreased with increasing birth order, with later born children more likely to have an intellectual disability. Compared to first-born children with siblings, first-born children without siblings at the time of diagnosis also exhibited decreased cognitive functioning. **Conclusions:** These findings demonstrate for the first time an association between increasing birth order and variability in ASD clinical phenotypes at diagnosis, with potential evidence of reproductive curtailment in children without siblings. Taken together, these findings have significant implications for advancing understanding about the potential mechanisms that contribute to heterogeneity in ASD clinical presentations as a function of birth order and family size. **Keywords**: Autism spectrum disorder; first birth; diagnosis; intellectual disability.

Introduction

The order of an individual's birth has been linked to a broad range of outcomes, ranging from intelligence (Kristensen & Bjerkedal, 2007; Rohrer, Egloff, & Schmukle, 2015), educational attainment (Barclay, 2015b, 2015a; Black, Devereux, & Salvanes, 2005), to

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sexual orientation (Blanchard, 2004; Bogaert, 2006). While explanations for these effects vary, a prominent hypothesis implicates the maternal prenatal immune environment influencing the developing brain (Balthazart, 2018; Careaga, Murai, & Bauman, 2017; Patterson, 2009). That is, a mother may develop an immunological reaction or antibodies to antigens during a pregnancy which may impact typical neurodevelopment, with this immune-mediated reaction becoming compounded or increasingly likely with each subsequent pregnancy. While some have argued that social rank within a family is more important than birth order *per se* (Kristensen & Bjerkedal, 2007), studies in adopted or non-biological siblings have indicated that biological birth order appears to exert a more substantial effect on outcomes; for example, with relation to sexual orientation (the 'fraternal birth order effect') (Bogaert, 2006). The potential impact of previous pregnancies on the developing brain has gained increasing attention, with recent emphasis placed on the relationship between birth order with neurodevelopmental outcomes, such as likelihood for an Autism Spectrum Disorder (ASD) diagnosis.

Birth order is one of a variety of population-level risk factors associated with increased odds of an ASD diagnosis (Gardener, Spiegelman, & Buka, 2009), with increased odds reported for both first-borns (Bilder, Pinborough-Zimmerman, Miller, & McMahon, 2009; Cheslack-Postava et al., 2014; Schmidt et al., 2013) as well as later births (Hultman, Sparen, & Cnattingius, 2002). Birth order relationships with ASD diagnoses may be moderated by family size; for example, individuals with ASD may be more likely first-born in smaller families with two children and born later in larger families (Lord, 1992; Tsai & Stewart, 1983). Further complex effects of birth order have been observed in recurrence rates, or the likelihood of another child in a family receiving an ASD diagnosis; Risch and colleagues (2014) reported in a large population-based data linkage study a significant increased rate of additional ASD diagnoses in second-born siblings relative to first-born children with a diagnosis, but conversely lower rates in third or later born siblings. Although any effects of birth order in ASD will be intrinsically linked to increasing parental age (Sandin et al., 2016), a well-documented factor associated with likelihood of an ASD diagnosis (Glasson et al., 2004), observed effects may also be confounded by so-called 'reproductive stoppage'; that is, parents delaying or not conceiving further children after one child has been diagnosed with ASD (Grønborg, Hansen, Nielsen, Skytthe, & Parner, 2015; Hoffmann et al., 2014; Kuja-Halkola et al., 2019). Recent evidence from a Swedish population registry study suggests that families with a first-born child diagnosed with ASD may have fewer subsequent children, whereas families with a second child or later-born

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child with ASD tend to have larger families, further underscoring a complex relationship between birth order and ASD diagnosis.

In parallel, preliminary evidence has observed associations between birth order and variability in ASD clinical phenotypes. In 1992, Lord first reported decreasing nonverbal IQ scores with increasing birth order in a small sample of multiplex families; that is, families with more than one child diagnosed with ASD (Lord, 1992). Subsequently, three clinical studies have converged on similar conclusions: ASD symptom burden increases commensurate with birth order in multiplex families (Martin & Horriat, 2012; Reichenberg, Smith, Schmeidler, & Silverman, 2007; Spiker et al., 2001). However, it is unknown whether these findings may be generalizable to the broader population of individuals diagnosed with ASD, most of which do not have a known family history of, or siblings diagnosed with, ASD. Given that parents may face decisions about conceiving further children after a child has received an ASD diagnosis, it is of clear importance to understand whether birth order may represent a potential mechanism that contributes to not just increased likelihood of ASD but to heterogeneity of clinical symptoms.

The present investigation is the first study to investigate the effects of birth order on diagnostic phenotypes in a large, population-based and representative sample of children diagnosed with ASD. We asked whether a child's level of symptom burden was associated with birth order utilizing data from a long-term prospective register in Western Australia. We predicted that increasing order of birth would be associated with a greater severity of phenotypic features (decreased adaptive functioning, decreased IQ) at the time of ASD diagnosis. We also predicted that increasing birth order would be associated with a greater proportion of cases with intellectual impairments and siblings diagnosed with ASD. Lastly, to test potential 'reproductive stoppage' effects (Kuja-Halkola et al., 2019), we predicted that first-born children without younger siblings at the time of diagnosis would experience a higher burden of symptoms and functional disability relative to first-born children with siblings.

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Methods

Sample

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The Western Australian Register for Autism Spectrum Disorders is an ongoing, independent, and prospective collection of data about ASD diagnoses in the Australian state of Western Australia, established in 1999 (Glasson, 2002). At diagnosis, clinicians submit information using a standardised notification form. Identifying information (name, date of birth, postcode) was included with consent. Where consent for identifiable information was not provided, de-identified clinical data was recorded with sex and year of birth only. Notification to the register was voluntary and, up until 2010, ascertainment was enhanced by supplemental annual reviews of missing cases at the four primary centres where most diagnostic assessments were conducted. Diagnostic information from any missed cases was added to the register without identifying details. Data collection was approved by the Princess Margaret Hospital Human Research Ethics Committee (294EP) and analysis approved by The University of Western Australia Human Research Ethics Committee (RA/4/1/9111).

Since 1997, Western Australia has utilized a standardized procedure for diagnosis of ASD and determination of eligibility for government-supported therapies (Glasson et al., 2008). For children under 12 years of age, the diagnostic assessment is performed by three health professionals (paediatrician/psychiatrist, psychologist, speech-language pathologist), and for adolescents and adults, by at least two professionals (clinical psychologist and paediatrician/psychiatrist, and speech-language pathologist, if required). A typical ASD diagnostic evaluation consists of a psychological observation of behavior and collecting information from informants to ascertain ASD-relevant behaviors during early childhood, a cognitive or developmental assessment to determine intellectual functioning, and a speech-language assessment to identify language impairments. A medical assessment by a paediatrician or psychiatrist is also required. Results from these assessments are then collated to confirm that criteria for ASD have been met to Diagnostic and Statistical Manual for Mental Disorders (DSM) criteria (APA, 1994, 2013).

All cases notified to the Autism Register between January 1st, 1999 and June 30th, 2017 were considered for inclusion for analysis (*n* = 5941). Cases were selected for inclusion if they were between 1-18 years of age at diagnosis and were either an only child or born fourth or earlier (see below for calculation of birth order groups), Year of birth was provided for all cases. However, as the exact date of birth was only provided in 51% of cases, possible multiple pregnancies with siblings were ambiguous, and these cases were excluded from analysis (*n* = 224). A further 313 cases were excluded if they did not have

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phenotypic data recorded on adaptive functioning, intellectual disability or IQ (see below), yielding a final sample of *n* = 5404.

Phenotypic data

All data was collected using a standardised record form completed by diagnosing clinicians. Demographic data included year of birth, sex, country of birth, mother's and father's ethnicity, and primary language at home. Parental ethnicity was reported as Caucasian, Asian, Aboriginal or Torres Strait Islander, or Other. Data about siblings were recorded by clinicians as sex, year of birth, relationship (full or half sibling), and ASD diagnosis (suspected or confirmed) for each sibling as reported by families at diagnosis. Only confirmed ASD in siblings was included in analysis of recurrence of ASD within families. Diagnostic information was coded as date of diagnosis and DSM diagnosis met (DSM-IV or DSM-5).

Adaptive functioning was measured using the Vineland Adaptive Behavior Scales (VABS), yielding an overall Composite score along with standard scores in Communication, Daily Living, Socialization, and Motor Skills (< 7 years of age, only) (Sparrow, Cicchetti, & Balla, 2005). Due to the wide range of years spanned by this study, the original Vineland scales, as well as the second edition (Vineland-II), in survey or interview versions were reported. Correlations between the first and second versions have been reported between 0.68-0.96 across domains/sub-domains and across ages (Sparrow, Balla, Cicchetti, Harrison, & Doll, 1984; Sparrow et al., 2005).

Intellectual disability (ID) was defined by the clinician's report of confirmed ID for DSM-IV cases, the DSM-5 specifier ('with intellectual impairment'), or, in the absence of these reports, an IQ (or Developmental Quotient) score below 70. Cases with global developmental delay or clinician's report of suspected ID were initially classed as 'Vulnerable for ID' and then re-classified as 'With ID', consistent with our previous analysis of this dataset (Alvares et al., 2020). Reclassifying 'vulnerable for ID' cases to ID was done as these children were too young at the time of diagnostic evaluation to receive an ID diagnosis, or where formal evaluation was not conducted, but were considered to be 'at risk' for ID by the clinician. Cases without IQ scores but with clinician indication of no intellectual difficulties were coded as 'No ID'. Cases without IQ scores or an indication of ID was classed as missing.

Statistical analysis

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All data were analysed using the statistical software *R* (R Core Team, 2019); packages are listed in Appendix S1. Birth order was calculated by ranking sibling's birth year against the case to establish order within the family. Although data about relationship to case (full or half sibling, or unknown) were recorded by clinicians, maternal or paternal relationships were not, and distinctions between maternal and paternal birth order were unable to be calculated; therefore, any sibling (full, half, or unknown) with data recorded was included in birth order derivation. The number of reported siblings ranged between 0 and 11, and birth order ranged from first to eighth born; birth order was calculated before multiple births were excluded from further analysis. Due to the small number of cases born fifth or later (<2% of total sample), cases were only included for further analysis for if their birth order was between first to fourth (first-, second-, third-, and fourth-born). The 'only child' group consisted of first-born cases where there were no recorded siblings at the time of diagnosis.

Multivariable linear regressions were used to determine whether birth order predicted VABS and IQ scores. Models were constructed for IQ scores and the VABS Composite score, as well as for the four domain scores (Communication, Daily Living, Socialization, and Motor Skills). Logistic regressions were used to test any associations between birth order and dichotomous outcomes (likelihood of having ID, recurrence of ASD in a sibling). Results from models are reported as effect estimates for continuous variables and odds ratios (OR) for categorical outcomes, with 95% confidence intervals (CIs). All models were adjusted for age and year of diagnosis (mean-centred), with first-born cases the reference group. Sensitivity analyses were conducted for all models to understand consistency of effects, first split by sex, second restricted to cases only with confirmed full biological siblings, and excluding multiplex cases with a sibling diagnosed with ASD. Exploratory analyses were also conducted to explore relationships between birth order and family size, with models split by family size (2 child, 3 child, and 4 child families), and only including cases with siblings.

Results

Sample characteristics

See Table 1 for sample characteristics by birth order (*n* = 5404). For the entire cohort, mean age at diagnosis was 6.6 (SD = 3.8) and 81.2% of the sample was male (*n* = 4374). Most spoke English as a first language, were born in Australia, and were ethnically

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Caucasian. The majority of the sample were diagnosed using DSM-IV criteria (Autistic Disorder *n* = 3390, Asperger's Disorder *n* = 212, PDD-NOS *n* = 588) with 21.6% diagnosed using DSM-5 criteria (Autism Spectrum Disorder *n* = 1156).

Adaptive functioning

After adjusting for age and year of diagnosis, relative to being first-born (in multiple child families), increasing birth order was associated with significant decreases in adaptive functioning scores; see Figure 1 and Table S1. On average, later born children had lower adaptive functioning Composite scores, which became more pronounced with increasing birth order. For example, second-borns on average had a 1.83 (95% CI: -2.93, -0.73) reduction in Composite scores relative to first-borns, third-borns a 2.51 reduction (95% CI: -4.06, -0.97), and fourth-borns a 4.28 reduction (95% CI: -6.60, -1.95); Table S1. Reductions in adaptive functioning by birth order were consistent across adaptive functioning domains (Communication, Daily Living Skills, Socialization, Motor Skills). In first-born cases without siblings at diagnosis ('only child'), lower Communication scores were observed relative to first-born children with siblings (-2.12, 95% CI: -3.65, -0.58). Adaptive functioning scores were negatively associated with age and positively associated with year of diagnosis, Table 2. Models analysed separately by sex indicated that significant associations between increasing birth order and decreases in adaptive functioning were consistent in male cases however there was insufficient evidence in female cases, likely attributable to reduced power in the latter analysis; Table S2. Models re-analysed including only cases with confirmed full-siblings or cases without siblings also yielded a similar pattern of findings, although effect sizes were reduced somewhat for the former sensitivity analysis; Tables S3-4 and Figure S1.

With respect to family size, significant reductions in adaptive functioning was observed in second-born cases, relative to first-borns, from two child families on Composite, Communication and Daily Living, but not for Socialization or Motor Skills domains, indicating broader consistency with the overall model. For example, second-borns exhibited a 2.05 reduction in Composite scores (95%CI: -3.37, -0.74) and a 3.97 reduction in Communication scores (95%CI: -5.69, -2.25), relative to first-borns. For later born cases, effect estimates were more inconclusive; for third-born cases in three child families, significant reductions in adaptive functioning scores were observed on Composite, Communication and Daily Living, but not for Motor Skills domains, and reduced but significant effect estimates for second-born cases in three child families on Communication

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and Socialization domains. Significant reductions in Socialization estimates were observed in both second and fourth born cases in four child families, albeit with very wide confidence intervals; Table S5.

Cognitive functioning

IQ scores were available for 27% (*n* = 1476) of the sample. Relative to first-borns, children without siblings had a decreased IQ of -4.52 points (95% CI: -7.53, -1.51), while second-born children had a decreased IQ of -3.82 points (95% CI: -6.53, -1.12); Table 2. Effects were broadly consistent in third-borns, but there was insufficient evidence for statistical significance in fourth-born cases.

Relative to first-borns, there were significantly higher odds of having ID in only-child cases (OR 1.40, 95% CI: 1.18, 1.66) and in fourth-borns (OR 1.50, 95% CI: 1.08, 2.08) cases; Table 2. Models split by sex suggest associations between decreased IQ and birth order were maintained in both sexes, however, the sample had only enough power to detect a statistically significant response in males; Table S2. Models re-analysed including only cases with full-siblings or cases without siblings diagnosed with ASD indicated a similar overall pattern, albeit with reduced magnitude of effects for the full siblings analysis; Table S3-4.

When analyses were split by family size, significantly reduced IQ (an average -4.79 points, 95% CI: -8.25, -1.32) and a small increased likelihood for ID (OR 1.28, 95%CI: 1.06, 1.54) were consistent in second-born cases in two child families, while effects in other birth order groups in three and four child families non-significant; see Table S5.

ASD recurrence in siblings

Of the sample with siblings, 10.05% (*n* = 428) had at least one sibling also diagnosed with ASD. As larger families are probabilistically more likely to have the opportunity to have more children diagnosed with ASD, the relationship between birth order and ASD recurrence was further explored, controlling for age, year of diagnosis, and size of family. Increasing birth order was associated with an increased likelihood of having a sibling diagnosed with ASD. For second-born children, relative to first-borns, there was a significantly increased likelihood of being in a multiplex family by an odds ratio of 2.36 (95% CI: 1.83 to 3.04; third-born 3.08 (95% CI: 2.24, 4.24), fourth-born 2.40 (95% CI: 1.44, 4.01); all $p<.001$). Family size (number of siblings) was also associated with a small increased likelihood of being in a multiplex family (OR 1.16, 95% CI: 1.01, 1.33).

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Discussion

The current study found support for the hypothesis that increasing birth order in children diagnosed with ASD was associated with increased functional and cognitive disabilities. Observed effects were influenced by family size, with significant effect estimates for adaptive behavior and cognitive impairments remaining for second-borns in two-child families, while effects in later birth orders in larger families did not reach statistical significance and were not as conclusive. Children without siblings at diagnosis also exhibited increased likelihood of cognitive impairments relative to first-borns with siblings. Previous findings have demonstrated effects of birth order on symptom profiles in multiplex families (Lord, 1992; Martin & Horriat, 2012; Reichenberg et al., 2007; Spiker et al., 2001); these findings are the first to confirm in a large and clinically ascertained population sample a potential association between increasing birth order and severity of ASD diagnostic phenotypes in children across the spectrum, with strongest effects observed with adaptive functioning. While all individuals diagnosed with ASD share broad clinical features to meet diagnostic criteria, extensive heterogeneity in clinical profiles are now considered hallmarks of the condition. This heterogeneity has hindered attempts to identify etiological mechanisms that may support the development of earlier identification and individualized intervention strategies (Masi, DeMayo, Glozier, & Guastella, 2017). The present findings shed light on birth order and family size as potential sources of variability contributing to clinical phenotypes that may inform such etiological research.

Increased adaptive functional impairments in later-born children and children without siblings suggest that birth order may influence the impact of autism diagnostic symptoms on an individual's ability to function in everyday environments. Adaptive functioning challenges in individuals on the autism spectrum are associated with educational attainment (De Bildt, Sytema, Kraijer, Sparrow, & Minderaa, 2005) and independent living outcomes (Farley et al., 2009), as well as requirements for support services (Taylor & Henninger, 2015). Large discrepancies between standardised adaptive functioning scores and IQ scores in children without intellectual impairments have also been described by our study team in a previous analysis of this WA Autism Register cohort (Alvares et al., 2020). In the present study, adaptive functioning scores across the composite and domains were significantly reduced

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between 2-5 points below the intercept for most birth order groups, relative to first-borns. When family size was considered, significant reductions were only observed in second-borns of two-child families in the order of 2-4 points below intercept for the Composite, Communication and Daily Living domains. Previous research in an ASD cohort has established that differences between 2-3.75 points on the Vineland scales represent a clinically important difference (Chatham et al., 2018). For example, with scores below 70 representing clinically significant scores requiring support, reductions in scores on the Daily Living domain in third and fourth born cases in the overall cohort resulted in scores falling in the clinically significant range relative to first-borns, although these did not achieve significance when considering third and fourth born cases within three and four-child family groups. Scores in the Communication domain were particularly impacted in the overall model, with significant reductions across all birth order groups relative to first borns, while the Socialization domain exhibited significant reductions in later-born cases in larger families. Although individuals on the autism spectrum typically exhibit most impaired scores on the Socialization domain (Kanne et al., 2011), these differing patterns of findings in the Communication and Socialization domains depending on family size may suggest that any potential associations between birth order and clinical phenotypes may be more complex, and possibly related to broader developmental delays, than specific ASD-related adaptive functioning difficulties.

Reproductive delays or stoppage may explain the finding of increased intellectual impairments in children without siblings relative to first-born children. This refers to the observed phenomenon of parents delaying or choosing not to have further children following developmental concerns in a child or a diagnosis of ASD. Curtailment in reproductive rates, or the reduced likelihood of having a subsequent child, has previously been reported in families of children with ASD (Hoffmann et al., 2014), but has not been linked to clinical phenotypes. More recently, likelihood of having additional children was reported in a large population Swedish sample to depend on birth order, with reduction in the likelihood of subsequent children only observed when a first-born child had an ASD diagnosis (Kuja-Halkola et al., 2019). Importantly, these findings did not appear to be due to changes in interpregnancy intervals in these families; both long and short interpregnancy intervals have been linked to the likelihood of an ASD diagnosis (Durkin, DuBois, & Maenner, 2015), and change in interpregnancy intervals is often taken as a measure for evaluating reproductive stoppage effects (Hoffmann et al., 2014). Given the cross-sectional nature of the dataset, we were unable to test hypotheses about likelihood or decision-

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making of families in having subsequent children, nor relationships to interpregnancy intervals. However, our additional observation that later-born cases were significantly more likely than first-born cases to have sibling recurrence of ASD may also suggest that reproductive curtailment or delays may be specific to families of children with more significant intellectual impairments. Subsequent analysis indicated this effect appeared to be specific to second-born children in two-child families, which may also be further evidence in support of potential reproductive stoppage in families of children with significant cognitive functioning impairments. Combined, these results provide the first evidence to suggest that severity of ASD diagnostic phenotypes, particularly intellectual and functional deficits, may be one of many factors, including family size, influencing reproductive decisions in families where a child has been diagnosed with ASD.

The observed findings of increased functional and cognitive impairments in later-born children with ASD may be explained by a range of biological or sociological theories. Maternal immune theories highlight the relationship between immune response to potential pathogens during pregnancy and subsequent brain development, with increased risk for ASD diagnoses in offspring whose mothers were hospitalised with infections during pregnancy (Jiang et al., 2016). For example, women caring for older children while pregnant may be exposed to a greater burden of infectious diseases (Marshall & Adler, 2009) or prenatal stress (Varcin, Alvares, Uljarević, & Whitehouse, 2017) which may impact subsequent brain development during pregnancy and subsequent phenotypes. The link between birth order and ASD may also be explained by factors related to fertility or pregnancy. Obstetric complications are more common in first- as well as later-born children, and this may increase both likelihood of ASD diagnosis and symptom severity (Bolton et al., 1997; Glasson et al., 2004; Zwaigenbaum et al., 2002). Additionally, as birth order and family size are positively associated with parental age, and increasing parental age has been strongly associated with ASD risk, it is possible that any effects of birth order on phenotype may be explained by advancing parental age (Bilder et al., 2009; Durkin et al., 2008; Sandin et al., 2016). However, to our best knowledge, no large studies have yet examined associations between parental age and variability in ASD phenotypes. A potential explanation for these findings may also relate to increase genetic liability for likelihood for ASD diagnosis in families that already have one child diagnosed with ASD, consistent with the first observations of birth order effects on IQ in multiplex families (Lord, 1992; Spiker et al., 2001). Sensitivity analyses suggested that findings remained consistent when excluding any cases with at least one sibling diagnosed with ASD. While this does not preclude

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genetic explanations for these findings, it may suggest that effects may be better explained by factors related to pregnancy, birth, or the environment. One alternative explanation may be that the experience of parenting and parent-child relationships may be different in later-born children or children without siblings relative to first-born children. As adaptive functioning estimates are primarily based on parental or carer report, these findings may reflect the parent's perception of phenotypic differences. Empirical testing of these hypotheses could be achieved in newly emerging and large detailed ASD biobanks to identify potential explanations underlying the present observed associations (Alvares et al., 2018; Feliciano et al., 2018; Loth et al., 2017).

Our study was limited by the availability of data recorded by clinicians at the time of ASD diagnosis. Half of cases included in the study did not have an exact date of birth recorded, and siblings only had year of birth recorded, thus limiting our ability to investigate the potentially influential factor of interpregnancy intervals on clinical phenotypes. Both short and long interpregnancy intervals have been associated with increased risk for ASD (Durkin et al., 2015) and further research investigating the link between intervals and variability in clinical phenotypes would be of significant interest to understand the potential mechanism contributing to this observed effect. We were also limited by the lack of data collected on other possible variables that could mediate the observed findings including, but not limited to, parental age, education levels or income, and birth complications or other obstetric factors. Second, birth order was calculated based on reported sibling relationships. As birth order derivations may have included paternal half siblings, rather than strictly reflecting maternal parity, biological explanations for these effects should be interpreted with caution. However, effect estimates were broadly consistent when analyses were restricted to cases with full siblings only, lending greater confidence to the potential generalisability of observed findings. The siblings reported in this analysis were also only those who had a year of birth, and the impact of miscarriages or stillbirths in influencing birth order effects, both on phenotype and likelihood for ASD, is unknown. We were also limited in the ability to evaluate the interaction of sex with birth order, due to the low number of females within the larger birth order groups, however sensitivity analysis indicated that effects were consistent in males. As an ASD diagnosis is influenced by sex, with females less frequently diagnosed, replication of these results for females on the spectrum is essential to determine consistency of effects between sexes. Additionally, the relatedness of cases to each other within the registry was unable to be ascertained. While identifiable information was captured in many cases for the purposes of contacting for future research,

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linking cases together in families without parental consent for identifying information for all cases could not be determined. As such, estimates of ASD recurrence may be underestimated, although are broadly in line with previous population-based estimates (Miller et al., 2019; Risch et al., 2014). Lastly, we were unable to confirm consistency of effects in later born cases in families of larger sizes. While effects of family size indicated a degree of consistency in effects in second-borns in two-child families, inconsistency of effects sizes observed in later born cases, relative to first-borns, in larger families may have been due to either a more complex relationship between birth order and clinical phenotypes in these cases, or a lack of statistical power, particularly for higher order births in four-child families. Future studies could utilize newly established large ASD cohorts (for example, Feliciano et al., 2018) to investigate potential associations between birth order and clinical phenotype in larger family sizes.

In conclusion, these results are the first to our knowledge to report associations in diagnostic phenotypes with birth order in a large clinical sample of children at the time of ASD diagnosis. We observed significant increases in clinical severity for later-born children and children without siblings relative to those born first. These findings have significant implications for advancing understanding of one mechanism contributing to clinical heterogeneity in ASD and may partially explain previous observations of reproductive delays in families of first-born children diagnosed with ASD.

Supporting information

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

Table S1. Associations between adaptive functioning by birth order.

Table S2. Association between adaptive and cognitive functioning by birth order and sex.

Table S3. Association between adaptive and cognitive functioning by birth order, cases with full siblings only.

Table S4. Association between adaptive and cognitive functioning by birth order, for cases without siblings diagnosed with ASD.

Table S5. Associations between birth order and family size on adaptive and cognitive functioning.

Figure S1. Adaptive functioning estimates by birth order, full siblings only.

Appendix S1. List of R packages used.

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Key points

- Birth order effects have been linked to a range of outcomes in the general population, including intelligence, educational attainment, and sexual orientation. Increased likelihood for an autism diagnosis has also been observed in first and later-born individuals.
- In this observational study of 5404 children from a diagnostic autism registry in Australia, we report that increasing birth order was associated with decreased functioning at the time of autism diagnosis.
- These findings shed light onto a potential mechanism contributing to variability in autism diagnostic clinical profiles.

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Table 1. Sample Characteristics by Birth Order

	Only Child *n* = 1146	First Born *n* = 1886	Second Born *n* = 1570	Third Born *n* = 609	Fourth Born *n* = 193	Total Sample *N* = 5404
Age at diagnosis, mean (SD)	5.82 (3.76)	7.29 (3.76)	6.30 (3.70)	6.75 (3.69)	6.55 (3.82)	6.64 (3.78)
Sex						
Female	207 (18.3)	345 (18.3)	319 (20.3)	116 (19.0)	25 (13.0)	1012 (18.8)
Male	924 (81.7)	1539 (81.7)	1250 (79.7)	493 (81.0)	168 (87.0)	4374 (81.2)
Number of Siblings [a]						
1	-	1376 (73.1)	1044 (66.5)	-	-	2420 (56.9)
2	-	387 (20.6)	411 (26.2)	444 (72.9)	-	1242 (29.2)
3	-	84 (4.5)	91 (5.8)	134 (22.0)	147 (77.0)	456 (10.7)
4	-	25 (1.3)	18 (1.1)	23 (3.8)	38 (19.9)	104 (0.0)
5	-	10 (0.5)	5 (0.3)	8 (1.3)	6 (3.1)	29 (0.0)
Diagnosis						
Autistic Disorder	740 (65.1)	1121 (59.9)	1022 (66.1)	389 (64.9)	118 (61.1)	3390 (63.4)
Asperger's Disorder	32 (2.8)	113 (6.0)	41 (2.7)	22 (3.7)	4 (2.1)	212 (4.0)
PDD-NOS	128 (11.3)	196 (10.5)	168 (10.9)	65 (10.9)	31 (16.1)	588 (11.0)
ASD	237 (20.8)	440 (23.5)	316 (20.4)	123 (20.5)	40 (20.7)	1156 (21.6)
Maternal ethnicity						
Caucasian	778 (78.4)	1483 (86.5)	1245 (86.0)	514 (89.2)	156 (86.7)	4176 (85.1)
Asian	130 (13.1)	128 (7.5)	115 (7.9)	33 (5.7)	11 (6.1)	417 (8.5)
Aboriginal	24 (2.4)	37 (2.2)	27 (1.9)	9 (1.6)	5 (2.8)	102 (2.1)
Other	60 (6.0)	66 (3.9)	60 (4.1)	20 (3.5)	8 (4.4)	214 (4.4)
Paternal						

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ethnicity						
Caucasian	768 (79.7)	1447 (86.2)	1214 (86.1)	503 (88.6)	154 (86.5)	4086 (85.2)
Asian	108 (11.2)	111 (6.6)	102 (7.2)	29 (5.1)	7 (3.9)	357 (7.4)
Aboriginal	18 (1.9)	38 (2.3)	28 (2.0)	10 (1.8)	6 (3.4)	100 (2.1)
Other	70 (7.3)	82 (4.9)	66 (4.7)	26 (4.6)	11 (6.2)	255 (5.3)
Language spoken						
English	996 (86.9)	1725 (91.5)	1450 (92.4)	578 (94.9)	187 (96.9)	4936 (91.3)
Other	150 (13.1)	161 (8.5)	120 (7.6)	31 (5.1)	6 (3.1)	285 (8.7)
Country of birth						
Australia	962 (89.8)	1683 (92.4)	1411 (92.7)	560 (93.6)	183 (95.3)	4799 (92.20)
Other	109 (10.2)	139 (7.6)	111 (7.3)	38 (6.4)	9 (4.7)	406 (7.80)
VABS, mean (SD)						
Composite	63.1 (11.4)	63.5 (12.5)	62.5 (11.4)	61.4 (11.7)	59.9 (9.9)	62.7 (11.8)
Communication	66.2 (14.9)	68.4 (15.9)	64.7 (14.6)	63.8 (13.4)	64.1 (13.2)	66.1 (15.0)
Daily Living Skills	66.5 (13.3)	66.6 (15.8)	65.9 (14.4)	63.9 (15.3)	61.6 (13.9)	65.9 (14.7)
Socialization	62.5 (10.8)	62.4 (12.4)	62.0 (10.7)	61.1 (10.7)	59.6 (9.3)	62.0 (11.3)
Motor Skills	74.5 (14.1)	73.9 (14.9)	74.1 (13.8)	72.2 (14.1)	70.1 (14.9)	73.8 (14.3)
IQ, mean (SD)	82.4 (20.1)	87.1 (21.5)	82.6 (22.1)	83.5 (20.8)	84.7 (18.3)	84.5 (21.3)

Note. [a] Cases with more than 5 siblings excluded here due to low frequency across groups. *PDD-NOS:* Pervasive Developmental Disorder – Not Otherwise Specified; *ASD*: Autism Spectrum Disorder; *VABS:* Vineland Adaptive Behavior Scale Unless otherwise indicated, data are expressed as number (percentage).

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Table 2. Associations Between Cognitive Functioning and Birth Order

	IQ *n* = 1476	ID *n* = 5404
(Intercept)	81.66 ***	1.06
	(78.80, 84.52)	(0.90, 1.25)
Only Child	-4.52 **	1.40 ***
	(-7.53, -1.51)	(1.18, 1.66)
Second Born	-3.82 **	1.24 **
	(-6.53, -1.12)	(1.06, 1.45)
Third Born	-3.00	1.23
	(-6.66, 0.66)	(0.99, 1.53)
Fourth Born	-2.13	1.50 *
	(-8.18, 3.93)	(1.08, 2.08)
Age	0.62 ***	0.82 ***
	(0.34, 0.90)	(0.80, 0.84)
Diagnosis Year	-0.20	1.00
	(-0.47, 0.07)	(0.99, 1.01)
R^2	0.02	0.12

Note: Models were adjusted for age and year of diagnosis (mean-centred), with first-borns as the reference group. Square brackets represent 95% confidence intervals. Values for Intellectual Disability (ID) analysis are log-odds ratio, values for IQ are effect estimates. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

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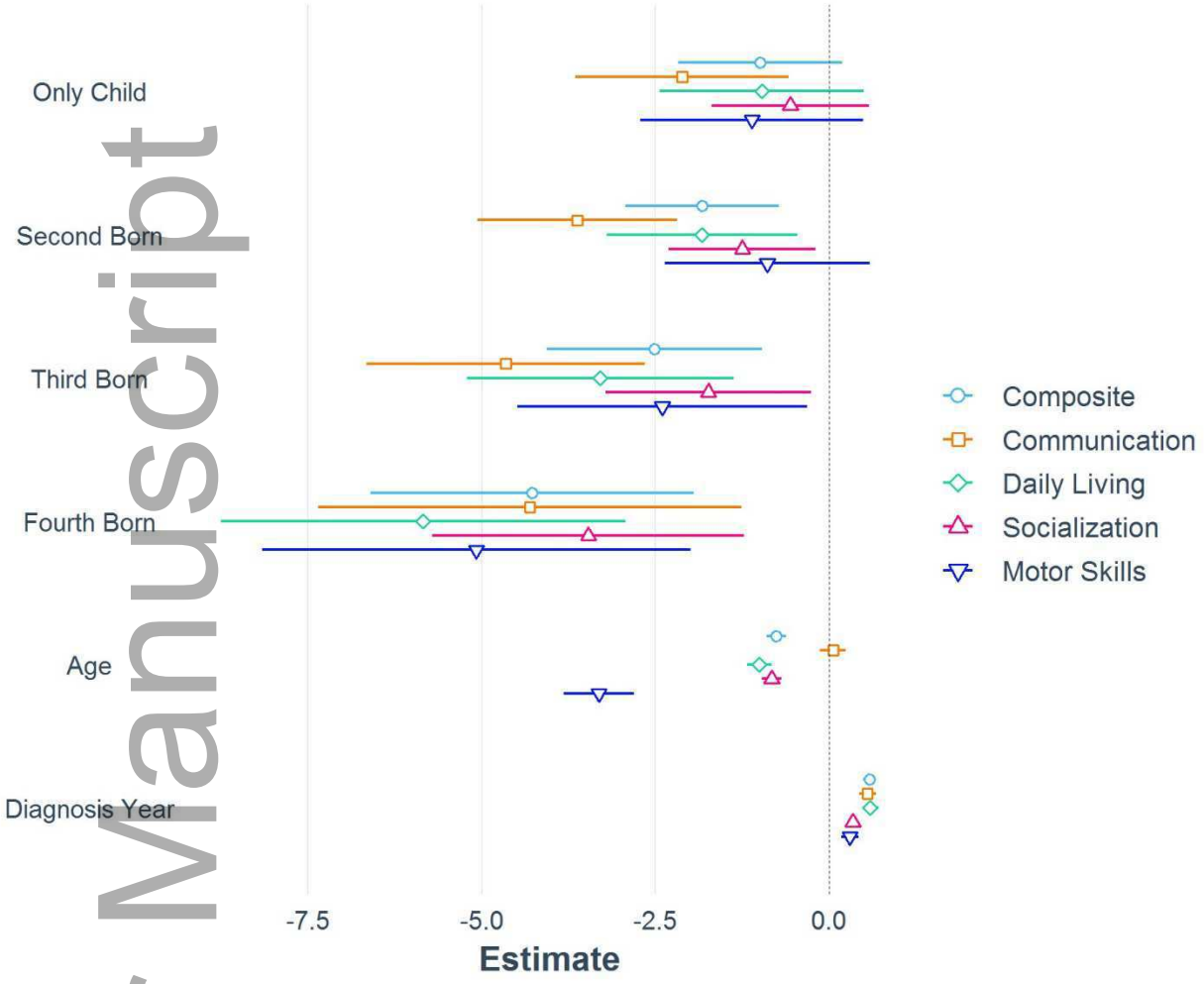
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Figure Captions

Figure 1. Adaptive Functioning Estimates by Birth Order.
Note: Points indicate effect estimates with horizontal lines indicating 95% confidence intervals; first-born cases were the reference group for all models.

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