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BRIEF REPORT

# Brief Report: Characteristics of preschool children with ASD vary by ascertainment

**Lori-Ann R. Sacrey[1,2] · Lonnie Zwaigenbaum[1,2] · Peter Szatmari[3,4,5] · Susan Bryson[6] · Stelios Georgiades[7] · Jessica Brian[3,8] · Isabel M. Smith[6] · Tracy Vaillancourt[9] · Nancy Garon[10] · Caroline Roncadin[3,11] · Mayada Elsabbagh[12]**

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**Abstract** Prospective studies of infant siblings of children diagnosed with autism spectrum disorder (ASD) provide a unique opportunity to characterize ASD as it unfolds. A critical question that remains unanswered is whether and how these children with ASD resemble other children identified from the community, including those with no family history. The purpose of this study was to compare clinical characteristics of children with ASD identified by each method (n = 86 per group), drawn from two Canadian longitudinal research cohorts. Children ascertained from a prospective cohort were less severely affected and included a larger proportion of girls, compared to the clinically referred sample. These results may have important implications for conclusions drawn from studies of high-risk and clinically referred cohorts.

**Keywords** Autism spectrum disorder · High-risk siblings · Prospective · Community referral · Comparison

## Introduction

Over the past decade, prospective high-risk designs have been employed to investigate early development in autism spectrum disorder (ASD), often with a focus on infant siblings of children with the diagnosis (Zwaigenbaum et al. 2009). Previous analyses of early parental concerns generated important insights that continue to inform the field provide unique opportunities to characterize ASD with respect to behavioural and brain development as it unfolds from the earliest months of life, to develop and evaluate intervention strategies based on these potential targets, and to map emerging features onto biological mechanisms (Jones et al. 2014).

However, a critical question that remains unanswered is whether and how children with ASD ascertained from prospective high-risk cohorts resemble other children with ASD; that is, children identified from the community, including those with no family history of ASD. Differences between the two groups may have important implications for the comparability of findings from high-risk cohorts to samples ascertained clinically. Critically, participants

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✉ Lori-Ann R. Sacrey
sacrey@ualberta.ca

1 Department of Pediatrics, University of Alberta, Edmonton, AB, Canada

2 Autism Research Centre—E209, Glenrose Rehabilitation Hospital, 10230-111 Avenue, Edmonton, AB T5G 0B7, Canada

3 University of Toronto, Toronto, ON, Canada

4 The Hospital for Sick Children, Toronto, ON, Canada

5 Centre for Addiction and Mental Health, Toronto, ON, Canada

6 Dalhousie University/IWK Health Centre, Halifax, NS, Canada

7 McMaster Univeristy, Hamilton, ON, Canada

8 Bloorview Research Institute, Toronto, ON, Canada

9 University of Ottawa, Ottawa, ON, Canada

10 Mount Allison University, Sackville, NB, Canada

11 McMaster Children's Hospital/Hamilton Health Sciences, Hamilton, ON, Canada

12 McGill University, Montreal, QC, Canada

in high-risk cohort studies generally undergo comprehensive diagnostic assessments regardless of symptom severity, whereas ascertainment of children with ASD in the community generally depends on clinical referral, the timing of which may be influenced by severity of symptoms and developmental delays (Daniels and Mandell 2013). As well, differences in biological factors (e.g., contribution of rare and common genetic variants, which may vary between single and multiple incidence families; Oerlemans et al. 2015, 2016) may affect the clinical profile associated with ASD.

The purpose of the present study was to examine the effect of ascertainment method on clinical characteristics of ASD in two Canadian samples. The availability of an inception cohort of newly diagnosed preschool children with ASD (the 'Pathways' study; Szatmari et al. 2015) provides a unique opportunity to assess phenotypic differences between community-referred children and those ascertained through a high-risk cohort. Children with ASD from the 'Pathways' study and an independent high-risk cohort were matched for age at diagnosis and their ASD symptoms and adaptive functioning were compared. It was predicted that children from the community-referred cohort would display higher ASD symptomatology and poorer adaptive functioning compared to the children ascertained from the high-risk cohort.

## Methods

### Participants

Children in the Pathways study [hereafter, 'Pathways cohort'] were recruited from the community following an initial diagnosis of ASD (in the previous 4 months), among children aged 2.0–4.11 years (Szatmari et al. 2015). Participants were assessed at clinical diagnostic centres in Vancouver, Edmonton, Toronto, Montreal, and Halifax, and met DSM-IV-TR criteria for ASD according to clinical judgment by a multidisciplinary team. Diagnoses were confirmed with the Autism Diagnostic Observation Schedule (ADOS; Lord et al. 2000) and Autism Diagnostic Interview-Revised (ADI-R; Lord et al. 1994). Families of all children given a diagnosis of ASD at one of these centres between June 2005 and May 2011 were approached to participate in the study. Of 728 children approached, 421 (355 boys; mean age at enrollment was 39.87 months) gave informed consent to participate (58%). The mean delay between receiving the diagnosis and enrolling in the study was about 1 month. Only one child per family was enrolled.

Children from the *Infant Sibling Study* [hereafter, 'Siblings cohort'] were recruited from 4 clinical diagnostic centres (Edmonton, Toronto, Hamilton, and Halifax) if the family had at least one child diagnosed with ASD and an infant aged between 6 and 12 months (Zwaigenbaum et al. 2005, 2015). ASD diagnoses were assigned between the ages of 18 and 36 months, when children met clinical criteria for the disorder as characterized by DSM-IV criteria according to clinical judgment by an expert diagnostician, informed by both the ADOS (Lord et al. 2000) and ADI-R (Lord et al. 1994). Based on the study design, age of diagnosis was largely constrained to age 2 years (23–26 months) and age 3 years (35–42 months, with efforts to assess as close as 36 months as possible). Diagnoses given at age 2 were confirmed at age 3.

The two groups were matched (1:1) for age at diagnosis (within 1 month) at age 2 or 3 years (eligible participants for Pathways cohort, n = 334, and Siblings cohort, n = 132). All of the children in the Siblings cohort had at least one older sibling diagnosed with ASD. In contrast, only 14% of the children in the Pathways cohort had any siblings diagnosed with ASD (as identified by parents in the most recent appointment up to age 8). There was no exclusion based on presence of affected siblings, but only one child per family was included in the study. For optimal comparability, the current study included children from both cohorts who were diagnosed at 2 or 3 years of age. Further comparisons beyond age 3 would have been ideal; however, follow-ups with families in the Siblings cohort beyond age 3 were less complete and thus, potentially less representative of the sample. As such, we were limited in the age range at which children from the two studies could be compared, specifically to ages 2 and 3 years.

The community sample as a whole received their diagnoses between the ages of 24 and 59 months of age (by study design), whereas the sibling sample received their diagnoses between 18 and 36 months of age. Our primary objective was to compare the features of children age 3 years or younger in the two studies, rather than age of diagnosis. Therefore; thus we matched by age, which reduced the total number of children from each study who contributed to the present study. For both studies, clinical diagnoses were established by experienced clinicians using DSM-IV-TR (the diagnostic framework in use at the time of data collection), based on developmental history (with symptom details from the ADI-R), observed behavioral symptoms (informed by the ADOS), and information about functional impairments. It is important to note that some of the same clinicians were involved in diagnosing children at the three overlapping sites (Edmonton, Toronto, and Halifax).

### Measures

Autism spectrum disorder symptoms and adaptive skills were measured using the Autism Diagnostic Observation

Schedule (ADOS), the Autism Diagnostic Interview-Revised (ADI-R), and the Vineland Adaptive Behavior Scales, 1st and 2nd editions (VABS and VABS-2).

*Autism Diagnostic Observation Schedule (ADOS; Lord et al. 2000)*

The ADOS uses standardized activities and 'presses' to elicit communication, social interaction, imaginative use of play materials, and repetitive behaviour. Inter-rater reliability of the ADOS is excellent (Lord et al. 2000). The scoring algorithm is organized into two domains, Social Affect (including Communication and Social items), and Restricted and Repetitive Behaviours (Gotham et al. 2007). The ADOS consists of four modules, each of which is appropriate for individuals of differing language levels (Module 1 = minimal or no language, Module 2 = regular use of non-echoed 3-word phrases, Module 3 = child with fluent language; and Module 4 = adolescent or adult with fluent language). The first three modules were used to assess participants in this study. To optimize comparability across modules, severity indices for Social Affect, Restricted and Repetitive Behaviour, and Total ADOS scores were calculated (Gotham et al. 2009). Note that the ADOS Toddler Module was not available when the data were collected.

*Autism Diagnostic Interview-Revised (ADI-R; Lord et al. 1994)*

The ADI-R is an investigator-directed interview that elicits information regarding social development, verbal and non-verbal communication skills, and the presence of repetitive, stereotyped interests and behaviour required to make an ICD-10 or DSM-IV-TR diagnosis of ASD. The questions are designed to distinguish qualitative impairments from developmental delays. The ADI-R consists of three domains: (a) reciprocal social interaction, (b) abnormalities in communication, and (c) restricted, repetitive, and stereotyped patterns of behaviours. A cut-off point for each of the three domains provides a reliable diagnostic algorithm shown to be accurate in differentiating autism from other developmental disorders (Rutter et al. 2003) and inter-rater reliability is excellent (Lord et al. 1994). The toddler algorithm, which classifies young children based on age and language level, was calculated for comparison between groups (Kim and Lord 2012). The ADI-R was administered at 36 months of age for the Sibling cohort; therefore, comparisons on this measure are only to those diagnosed at age 3 in the Pathways cohort.

*Vineland Adaptive Behavior Scales, 1st and 2nd ed. (VABS; Sparrow et al. 1984, 2005)*

The VABS is a semi-structured parent interview designed to assess adaptive behaviour in four domains—Communication, Daily Living, Socialization, and Motor skills (limited to children younger than 6 years), outlined by typical developmental milestones that are anchored to specific ages. The scale has excellent reliability and concurrent validity, and is sensitive to impairments experienced by children with ASD (Volkmar et al. 1993; Carter et al. 1998). VABS scores from 24 to 36 months were compared. The Siblings cohort used the first edition of VABS and the Pathways cohort used the second edition (VABS-2), scores were categorized for level of adaptive function (by domain) of children who scored in the impaired (standard scores fell in low and moderate low [standard score $\leq 85$]) versus average range (standard scores fell between adequate and high [standard score $\geq 85$]; Sparrow et al. 1984).

### Statistical Analyses

Clinical characteristics of the groups were compared using the Statistical Package of the Social Sciences (SPSS v.19). A multivariate analysis of variance (MANOVA) was performed to test for overall differences between groups (Sibling vs. Pathways cohorts), sex (boy vs. girl), age at diagnosis (2 vs. 3 years), as well as interactions of group by sex and group by age at diagnosis. Groups were then compared at each age at diagnosis on the ADI-R and ADOS subdomain scores to unpack interaction effects using MANOVAs. Statistical significance was set at alpha $\leq 0.05$ and significant effects were explored using Bonferroni correction.

## Results

### Participants

Children were matched 1:1 based on age of diagnosis (within one month), resulting in the inclusion of 172 children in the study; 86 children (58 boys and 28 girls) from the Sibling cohort and 86 children (73 boys and 13 girls) from the Pathways cohort. Additional children were diagnosed around age 2 or 3 years, however they did not match on age at diagnosis and thus were not included. There was a significant between-groups difference ($\chi^2(1) = 7.21$, $p = .007$), with a higher proportion of girls in the Sibling cohort than the Pathways cohort. Overall, 41 children in each group were diagnosed with ASD at approximately age 2 years and 45 children at age 3 years. As the groups were matched for age at diagnosis, there were no group differences for actual age at the two-year assessment

($t(80) = 1.29$, $p = .20$; Siblings = 24.78 ± 2.53 months and Pathways = 25.46 ± 2.26 months) or three-year assessment ($t(88) = 0.36$, $p = .72$; Siblings = 37.07 ± 3.42 months and Pathways = 37.32 ± 3.37 months).

Comparisons of family demographics are displayed in Table 1. There was a significant group difference for both mothers' ($t(155) = 3.2$, $p < .001$) and fathers' ($t(150) = 3.7$, $p < .001$) ages at child's birth, with both parents being older in the Siblings cohort. There were significant group differences for marital status (Fishers Exact test = 9.0, $p < .05$), with the Sibling cohort reporting a higher proportion of two-parent families; maternal education (Fisher Exact Test = 20.6, $p < .001$), with mothers from the Pathways cohort reporting a higher proportion of 'some college'; and paternal education (Fisher Exact Test = 22.7, $p < .001$), with fathers from the Pathways cohort reporting a higher proportion of 'some college' and lower proportion of 'undergraduate degree.' There were no differences for ethnicity of the mother ($p = .07$) or father ($p = .16$). Comparisons of children who were included versus excluded for each study did not result in any significant differences.

The children with ASD from the two cohorts differed not only by ascertainment but also by whether or not the child was the only child with ASD in the family; all children with ASD in the high-risk cohort by definition were multiplex, whereas the majority of the community sample was simplex. Thus, we also compared features of simplex (n = 74) versus multiplex (n = 12) children *within* the community sample. There were no significant differences in ADOS severity scores, ADI-R domain scores, or proportion of children scoring within the adequate range on the VABS, at ages 2 and 3 years (p-values 0.33–1.00; effect sizes < 0.3; details available in supplemental material).

## Overall MANCOVA

A statistically significant overall MANOVA (controlling for sex) was observed for group (Pathways vs. Siblings;

**Table 1** Family demographics for the Pathways and Siblings cohorts

| | Pathways | | Siblings | |
|---|---|---|---|---|
| | n | % | n | % |
| Married/common-Law | 77 | 89.5 | 65 | 75.6 |
| Divorced/separated | 0 | 0.0 | 3 | 3.5 |
| Single mother | 2 | 2.3 | 10 | 11.6 |
| Unknown | 7 | 8.2 | 8 | 9.3 |
| Significance | *Difference between groups ($p < .05$) | | | |
| | Mother | Father | Mother | Father |
| Age at birth (years) | 32.4 (5.8) | 34.6 (6.6) | 35.1 (4.0) | 38.3 (5.9) |
| Significance | *Differences for both parents ($ps < 0.001$) | | | |
| | % | % | % | % |
| Caucasian | 75.6 | 66.3 | 55.8 | 64.0 |
| Asian | 4.7 | 7.0 | 7.0 | 4.7 |
| Aboriginal | 1.2 | 0.0 | 0.0 | 2.3 |
| East Indian | 2.3 | 2.3 | 2.3 | 1.2 |
| Latin-American | 1.2 | 1.2 | 1.2 | 0.0 |
| Middle Eastern | 2.3 | 1.2 | 5.8 | 3.5 |
| Other | 2.3 | 2.3 | 12.8 | 10.5 |
| Unknown | 10.5 | 19.8 | 15.1 | 14.0 |
| Significance | *No difference for ethnicity ($p > .05$) | | | |
| | % | % | % | % |
| < High school | 5.8 | 7.0 | 1.2 | 5.8 |
| Complete high school | 10.5 | 4.7 | 15.1 | 11.6 |
| Partial college/specialized training | 29.1 | 34.9 | 9.3 | 14.0 |
| Undergraduate degree | 27.9 | 17.4 | 44.2 | 40.7 |
| Graduate degree | 11.6 | 14.0 | 16.3 | 17.4 |
| Unknown | 15.1 | 22.1 | 14.0 | 10.5 |
| Significance | *Differences for both parents ($p < .001$) | | | |

$F$ (15, 126) = 17.71, $p < .001$; Wilk's $\Lambda = 0.32$), age at diagnosis (2 vs. 3 years; $F$ (15, 126) = 5.62, $p < .001$; Wilk's $\Lambda = 0.40$), and group by age at diagnosis interaction ($F$ (15, 126) = 2.76, $p < .001$; Wilk's $\Lambda = 0.75$) when comparing the dependent variables listed in Table 2. There was no main effect of sex ($F$ (15, 126) = 0.89, $p = .58$) or group by sex interaction ($F$ (15, 126) = 0.64, $p = .83$) for any of these variables. The effects of family demographics on the clinical scores were assessed and are reported in the supplementary material.

## Children diagnosed at age 2 years

Comparing the two groups on all measures together resulted in a significant overall MANCOVA for group ($F(15,50) = 11.14$, $p < .001$, Wilk's $\Lambda = 0.23$). Group comparisons on ADOS are described below and the results are summarized in Table 2. Percentages of children in each group who scored within the 'adequate adaptive level' or higher on VABS domain scores are also described below.

### *ADOS*

For children diagnosed at age 2 years, a significant between-groups difference was found for the Social-Affect severity score ($F(1, 73) = 3.91$, $p < .05$), but not for the Restricted Interests and Repetitive Behaviour severity score ($F(1, 73) = 0.006$, $p = .94$) or Total severity score ($F(1, 73) = 0.41$, $p = .52$). Children in the Sibling cohort had lower (i.e., fewer signs of atypicality) Social-Affect severity scores than children in the Pathways cohort.

### *VABS*

The percentage of children scoring in the adequate level or above range was higher for the Sibling cohort compared to the Pathways cohort for the domain scores of Communication (39.0% vs. 9.8%; $\chi^2$ (1) = 10.78, $p = .001$), Daily Living Skills (34.1% vs. 9.8%; $\chi^2$ (1) = 8.08, $p = .004$), and Socialization (36.6% vs. 17.1%; $\chi^2$ (1) = 4.78, $p = .029$), but was comparable for Motor skills (53.7% vs. 53.7%; $\chi^2$ (1) = 0.079, $p = .78$). Thus, children in the Sibling cohort were less likely to have adaptive impairments as indexed by three of the domain scales.

**Table 2** Characteristics of the children divided by group and age at diagnosis

| | Diagnosis at 2 years | | | | Diagnosis at 3 years | | | |
|---|---|---|---|---|---|---|---|---|
| | Sibling Mean (SD) n = 41 | Pathways Mean (SD) n = 41 | *F or X* | *p* | Sibling Mean (SD) n = 45 | Pathways Mean (SD) n = 45 | *F or X* | *p* |
| ADOS | | | | | | | | |
| SA | 6.13 (1.74) | 6.95 (1.82) | 3.91 | 0.05* | 6.07 (1.44) | 7.58 (1.79) | 19.26 | 0.001* |
| RRB | 8.00 (1.52) | 7.97 (1.55) | 0.01 | 0.94 | 7.31 (2.21) | 7.44 (1.80) | 0.09 | 0.76 |
| Total | 6.87 (1.92) | 7.14 (1.67) | 0.41 | 0.52 | 6.20 (1.38) | 7.40 (1.69) | 13.28 | 0.001* |
| ADI-R | | | | | | | | |
| SA | – | – | – | – | 5.39 (3.93) | 11.40 (3.88) | 51.14 | 0.001* |
| RRB | – | – | – | – | 2.59 (2.28) | 5.98 (2.87) | 36.97 | 0.001* |
| Play | – | – | – | – | 2.57 (2.34) | 6.74 (3.34) | 45.30 | 0.001* |
| Total | – | – | – | – | 10.55 (7.07) | 24.12 (7.27) | 70.07 | 0.001* |
| VABS / VABS-2[$] | | | | | | | | |
| Comm | 39.0% | 9.8% | 10.78 | 0.001* | 75.0% | 20.5% | 30.58 | 0.001* |
| DL | 34.1% | 9.8% | 8.08 | 0.004* | 43.2% | 18.2% | 9.29 | 0.002* |
| Social | 36.6% | 17.1% | 4.78 | 0.029* | 50.0% | 4.5% | 27.85 | 0.001* |
| Motor | 53.7% | 53.7% | 0.079 | 0.78 | 54.5% | 31.8% | 6.01 | 0.014* |

The standard deviations of the severity metrics of the ADOS and ADI-R are similar, suggesting the groups show comparable variability, despite mean group differences

*ADI-R* Autism Diagnostic Interview-Revised, *ADOS* Autism Diagnostic Observation Schedule, *Comm* Communication Domain, *DL* Daily Living Skills Domain, *Motor* Motor Domain, *Play* Imitation, Gestures & Play or Reciprocal and Peer Interaction Severity Metric, *RRB* Restricted Interests and Repetitive Behaviours Severity Metric, *SA* Social Affect Severity Metric, *SD* standard deviation, *Social* Socialization Domain,

*Significant at $p < .05$; VABS = Vineland Adaptive Behavior Scales;

[$]Percentage of children in each group who fell within the adequate or above range on the VABS/VABS-2

### Children Diagnosed at Age 3 Years

Comparing the two groups on all measures together resulted in a significant overall MANCOVA for group ($F(15,61) = 12.29$, $p < .001$, Wilk's $\Lambda = 0.25$). Group comparisons on ADOS and ADI-R are described below and the results are summarized in Table 2. Percentages of children in each group who scored within the 'adequate adaptive level' or higher on VABS domain scores are also described below.

#### *ADOS*

The Social-Affect severity score ($F(1, 87) = 19.26$, $p = .001$) and the Total severity score ($F(1, 87) = 13.28$, $p = .001$), but not the Restricted Interests and Repetitive Behaviour severity score ($F(1, 87) = 0.092$, $p = .76$), differed significantly between groups for children diagnosed at age 3 years. Children in the Sibling cohort had lower Social-Affect and Total severity scores (i.e., behaviour that was less atypical) than children in the Pathways cohort.

#### *ADI-R*

A group difference was observed for the ADI-R toddler algorithm Total score (including Social Affect, Repetitive & Restricted Behaviours, and Play subscales) for children diagnosed at age 3 ($F(1, 84) = 77.07$, $p = .001$). There were group differences for Social Affect ($F(1, 84) = 51.14$, $p = .001$), Repetitive & Restricted Behaviors ($F(1, 84) = 36.97$, $p = .001$), and Imitations, Gestures & Play/Reciprocal and Peer Interactions ($F(1, 84) = 45.30$, $p = .001$) domains of the ADI-R. Children in the Sibling cohort had lower overall scores and lower scores (i.e., behaviour that was reported to be less atypical) on each domain compared to children in the Pathways cohort.

#### *VABS*

The percentage of children scoring in the adequate level or above range was higher for the Sibling cohort compared to the Pathways cohort for the domain scores of Communication (75.0% vs. 20.5%; $\chi^2$ (1) = 30.58, $p = .001$), Daily Living Skills (43.2%vs. 18.2%; $\chi^2$ (1) = 9.29, $p = .002$), Socialization (50.0% vs. 4.5%; $\chi^2$ (1) = 27.85, $p = .001$), and Motor skills (54.5 vs. 31.8%; $\chi^2$ (1) = 6.01, $p = .014$). Thus, children in the Sibling cohort had more advanced adaptive skills as indexed by the all of the domain scales compared to children in the Pathways cohort.

## Discussion

In the present study, we compared the clinical characteristics of children diagnosed with ASD who were ascertained either as an infant sibling of a child diagnosed with ASD, or by community referral. Three important group differences were found: children recruited via community referral had (1) higher scores on the ADOS (at ages 2 and 3) and the ADI-R (available at age 3 only), indicating a greater degree of ASD symptomatology; (2) lower adaptive skills as indexed by the VABS; and (3) included a lower proportion of girls compared to children recruited via the Sibling study. These results suggest that ascertainment method can influence the clinical profiles observed in samples of children with ASD at the time of diagnosis.

Children with ASD identified from high-risk cohorts, as in the *Infant Sibling Study*, are generally enrolled between 6 and 12 months of age and are seen repeatedly during their first 3 years. These children were closely monitored regardless of clinical symptomatology, allowing for the earlier identification of subtle impairments or differences. This is in contrast to children with ASD participating in the Pathways study, who were referred by community professionals, and in whom queried symptoms were interfering with children's functioning. In a recent systematic review, Daniels and Mandell (2013) reported that less severe ASD symptoms and cognitive impairment, as well as behavior problems not perceived as specific to ASD (e.g., emotional outbursts) were associated with later age of diagnosis, which remains, on average at age 4 years or later in community samples. Daniels and Mandell (2013) also reported that socio-demographic factors including African-American and Latino ethnicity, lower parental income and education, and rural residence may also contribute to later diagnosis, presumably in part on the basis of later clinical referral, although these factors varied among reviewed studies. It is important to note that similar to previous reports, we did not find group differences for RRBs on the ADOS at 24 and 36 months (Kim and Lord 2010; Lord et al. 2000). This is not surprising as observational assessments occur within a brief period and may not be optimal for capturing RRBs. In contrast, parents observe their child on a daily basis and are more likely to observe and report on the presence of RRBs.

The Sibling cohort contained a higher proportion of girls diagnosed with ASD, compared to the Pathways cohort. This finding is consistent with previous reports of sex ratios in earlier versus later born children within multiplex families, i.e., ratios of 2.9:1 for later born versus 4.7:1 for first born children (Jones et al. 1996). It is also possible that higher functioning girls are under-ascertained in the community. For example, a recent report exploring the relation between sex, verbal ability, and age of diagnosis in a large community sample found that girls with more complex

speech were diagnosed later than boys with similar levels of verbal ability (Salomone et al. 2016). This finding is corroborated by secondary analyses of the data from the Autism and Developmental Disabilities Monitoring Network in the US, which found that girls were less likely to be diagnosed with ASD in a community setting, even though they met criteria on independent educational and developmental file review (Giarelli et al. 2010). Similarly, retrospective analysis of data from the Avon Longitudinal Study of Parents and Children noted an under-identification of girls compared to boys, even when symptom severity was held constant (Russel et al. 2011). This is echoed in a survey of males and females with ASD in the Netherlands, which found that the average age at diagnosis was older for girls with any variant of ASD (including Asperger syndrome) than for boys with comparable diagnoses (Begeer et al. 2013). Interestingly, there were no overall sex or group by sex differences in the present study that would have accounted for the higher ADOS and ADI-R scores and lower VABS scores in the Pathways cohort.

These findings highlight the importance of considering ascertainment in interpreting the results of early detection research in ASD. Children ascertained from high-risk samples (e.g., younger siblings of diagnosed children) may display fewer or less severe ASD symptoms and better adaptive skills than children from community referral. To the extent that early behavioural and biological markers of ASD vary by such indices at the time of diagnosis (e.g., 6- and 12-month behavioural markers may be more pronounced in children with milder ASD symptoms at diagnosis; Estes et al. 2015), caution should be exercised in generalizing findings from high-risk cohorts to community samples and vice versa. That said, a recent report comparing the cognitive profiles of probands from simplex versus multiplex families found no differences on measures of verbal and performance IQ, face recognition, identification of facial emotion, affective prosody, inhibition, verbal working memory, visual working memory, or set shifting errors (Oerlemans et al. 2016). A comparison of the children from simplex and multiplex families within the Pathways cohort reported here echo the absence of differences between these two groups. Nevertheless, it is possible that high-risk findings may be conservatively biased, whereby associations between early markers and later diagnosis are attenuated by more complete ascertainment (i.e., including those with milder symptoms, who are less likely to be clinically referred) of ASD cases at age 3.

Differences in developmental profile, symptom severity, and sex ratio among the children with ASD in the two cohorts have important implications. Children with milder symptoms and less severe language and cognitive impairments ascertained from the Sibling cohort may have a more favorable prognosis and potential for more robust response to intervention. Despite this, if living in jurisdictions where eligibility is contingent on severity of clinical presentation, these children may have less access to specialized treatment services. The two cohorts, due to differences in sex ratio and cognitive profiles, may also experiences different rates of mental health comorbidities over time. These possibilities will be important to evaluate by further longitudinal follow-up of these cohorts.

This study is not without limitations. Although data from both the Pathways and Siblings cohorts were collected from many of the same sites, there is only a partial overlap in clinicians performing the diagnostic assessments, and data collection occurred during different time periods. In addition, due to the nature of the Siblings cohort, we were only able to include children diagnosed at (or near) 2 and 3 years of age, thereby excluding the children diagnosed outside of these time windows. Furthermore, our analyses were limited to measures that overlapped between the two cohorts, thus we were unable to look at language, cognitive function, and comorbid emotional-behavioural symptomology. Importantly, our groups differed on family demographics, particularly marital status, parental education, and parental age at children's births. These factors could have impacted diagnoses and access to medical care for children. Similarly, social factors may have influenced the determination of diagnoses that may differ between the community referred sample and the high-risk sample. This limitation is at least partially mitigated by the confirmation of diagnoses in the community sample by the research team following study enrolment. Nevertheless, we were able to match 86 children per group based on age at diagnosis, and the data suggest that children ascertained from the Siblings cohort were less severely affected based on both measures of ASD symptoms and adaptive function compared to the clinically referred sample. Finally, and critically, although children participating in the Pathways cohort were diagnosed based on community referral, the study participation rate was only 58%, and factors associated with participation are not known. Moreover, participation biases may have varied between the Pathways and Siblings cohorts, contributing to group differences. it is unlikely that differences in exclusion criteria played any role in influencing the results since these occurences were rare, or no one in the Sibling cohort was not excluded because of extreme prematurity etc.

Ultimately, ascertainment is only one of many factors that contribute to clinical diversity within ASD, emphasizing the importance of considering individual differences when developing early detection and intervention strategies for children across the spectrum. No sampling strategy is free of bias; the critical reader must always take ascertainment method into account when interpreting the results of a

study, and understanding the variation is clinical characteristics associated with variation in ascertainment is therefore important.

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**Compliance with Ethical Standards**

**Conflict of interest** All of the authors declare that they have no conflicts of interest.

**Ethical approval** All of the procedures involving our human participants were performed in accordance with the ethical standards of the institutional boards at each institution and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants (parents) prior to study onset.

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