OBJECTIVE: This study aimed to determine the prevalence of autism spectrum disorder (ASD) by using the Autism Diagnostic Observation Schedule-Generic (ADOS-G) classifications in children born very preterm during their toddler years.

METHODS: Two birth cohorts of toddlers (2 and 4 years old) each recruited over 12 months and born at <29 weeks' gestation were administered the Modified Checklist of Autism in Toddlers–Follow-up Interview (M-CHAT-FI) screen, the ADOS-G, and developmental assessments. The ADOS-G was conducted on toddlers with M-CHAT-FI–positive screens.

RESULTS: Data were available on 88% (169/192) of children. In total, 22 (13%) toddlers screened positive and 3 (1.8%) were confirmed diagnostically with ASD. These 3 cases reached the highest ADOS-G threshold classification of autism. All but 1 child who scored below the ADOS-G thresholds (11/12) demonstrated some difficulty with social communication. Risk was significantly increased for co-occurring neurodevelopmental problems in 21 of the 22 positive-screen ASD cases. Adaptive behavior ($P < .001$) was the only co-occurring factor independently predictive of ASD in toddlers.

CONCLUSIONS: Children born very preterm are at increased risk of ASD. By using the ADOS-G, we found a lower incidence of ASD in children born at <29 weeks' gestation compared with previous studies. Children who screened positive for ASD on the M-CHAT-FI had developmental delays consistent with subthreshold communication impairment.
Autism spectrum disorders (ASDs) are a heterogeneous group of developmental disorders evident in early childhood and are characterized by qualitative impairments in communication, social relations, and repetitive and restricted behaviors and interests. When considering the global burden of disease, ASDs are the leading cause of disability in children <5 years of age.1,2 The diagnostic prevalence of disorders within the spectrum is reported to be higher in those born extremely3,4 and very preterm5–9 (3.6%–12.9%) compared with the general population prevalence (~1%).10 The rates for ASD are reported to be highest in 2-year-olds (12.9%)7 and similar in children (3.6%–8%)5,6,8 and adults (5%)9 born prematurely. The most commonly reported diagnostic classification within the spectrum in these studies is the narrowly defined autistic disorder (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition)11 or childhood autism (International Classification of Diseases, 10th Revision).12 High rates of co-occurring severe neonatal brain abnormalities and neurosensory and cognitive impairments also have been reported in children born preterm with ASD and are speculated to be implicated in the expression of the behaviors displayed.3,4,6 These impairments also are associated with the high positive screening rates reported in the preterm population.5,8,13–16 Although these screening studies overestimate diagnostic outcome, children born preterm often demonstrate a profile of early social communication and emotional difficulties consistent with ASD characteristics.4,14

All of the diagnostic studies reporting the prevalence of ASD in school-aged children used semistructured diagnostic interview assessment tools.3,4,6,8,9 Two studies used the direct semistructured observational assessment called the Autism Diagnostic Observation Schedule–Generic (ADOS-G) in toddlers7 or in combination with the Autism Diagnostic Interview-Revised17 in adults.5 A recent systematic review assessing the accuracy of diagnostic tools with the gold standard (multidisciplinary team assessments with consensus clinical judgment) diagnosis showed that the ADOS most reliably meets the threshold for clinical accuracy,18 which is further improved when children are assessed within the context of a neurodevelopmental clinic.19 In view of these reports, and the paucity of diagnostic studies using the ADOS-G, our primary aim was to determine the prevalence of ASD in the context of routine developmental follow-up for toddlers born very preterm. All children were screened by using the Modified Checklist of Autism in Toddlers–Follow-up Interview (M-CHAT-FI) and positive cases were diagnostically assessed using the ADOS-G. Secondarily, we examined the subthreshold autistic traits, and neonatal, family, and developmental characteristics associated with positive screening.

METHODS

The cohort comprised all infant survivors with their estimated date of confinement falling in 2006 and 2008, in children born at <29 weeks’ gestational age (GA), who received tertiary-level neonatal care at the Royal Brisbane and Women’s Hospital, Australia. Participants enrolled in the study were assessed at 2 and 4 years’ corrected age in conjunction with their routine developmental follow-up assessment in 2010. The study was approved by the institutional Human Research Ethics Committees. Parental informed written consent was required for participation.

Assessments and Measures

Autism study Assessments

Autism study assessments included the M-CHAT,20 which is a parent-completed 23-item yes/no screening tool for ASD. A positive ASD screen requires the child to be positive on any 3 items or any 2 of 6 critical items. Items that screened positive are further systematically probed with the FI. The FI follows a script that asks for specific examples of behaviors and offers multiple examples against which to judge whether the child passed or failed the item. The FI is reported to substantially improve the M-CHAT’s positive predictive value in the 2-year-old (from 0.36 to 0.74) and in the 4-year-old (from 0.38 to 0.59) general pediatric population.21 Children who screened M-CHAT-FI positive were assessed for ASD by an experienced educational psychologist trained in using the ADOS-G.22

The ADOS-G is the only tool that provides a semistructured direct assessment of the child’s social and communication skills and behavior. It comprises 4 modules based on verbal skills and is designed for use across age ranges from 2 years to adulthood. In this study, Module 1 (for children who do not consistently use phrased speech) and Module 2 (for children who use phrased speech but who are not verbally fluent) were used. Children with profound intellectual or motor disabilities fall below the floor of the measure and cannot be reliably assessed. Scoring is based on observations of the child’s behavior and items are scored for the communication and social interaction domains as well as for stereotypical behaviors and restricted interests, including play and imaginative use of materials. Each item is scored from 0 (no abnormality) to 2 or 3 (moderate...
to severe abnormality). Scores are placed into a diagnostic algorithm that gives separate domain scores for communication (range 0–10) and reciprocal social interaction (range 0–14), and a communication + social interaction total (range 0–24). Cutoff scores for both communication and reciprocal social interaction and their sum (Module 1: autism cutoff = 12; autism spectrum = 7; Module 2: autism = 12; autism spectrum = 8) are used to classify ASD as autism (highest cutoff score) and autism spectrum (lower cutoff score with fewer symptoms). The neurodevelopmental team evaluated all cases in the context of the child’s overall developmental assessment for potential misclassification.

**Developmental Follow-up Assessment**

Developmental follow-up assessment was routinely conducted on all children by a neurodevelopmental team blinded to the child’s history. Children were assessed for cerebral palsy, which included a neurologic examination and motor assessment, and for impairments in vision, visual acuity <6/60, and hearing, requiring aids. Cognitive and language ability were assessed by using the Bayley Scales of Infant and Toddler Development, Third Edition cognitive and language composite indices and the Wechsler Preschool and Primary Scale of Intelligence–Third Edition (Australian), Full-Scale IQ, and the Verbal IQ scores, in the 2- and 4-year-old children, respectively. Adaptive behavior was assessed by using the Adaptive Behavior Assessment System–Second Edition General Adaptive Composite score. Cognitive, language, and adaptive behavior were classified relative to the tests’ norms (mean = 100; SD = 15) for all assessments except the Bayley Scales of Infant and Toddler Development, Third Edition, in which Australian term-born reference data were used (Cognition, mean = 108.9; SD = 14.3; Language, mean = 108.2; SD = 14.8). Scores < –2 SDs were classified as moderate-severe delay. We used the corresponding domains on the Parents’ Evaluation of Developmental Status: Developmental Milestones–Assessment Level (PEDS:DM-AL) to assess cognition, language, and adaptive behavior in children who did not return for psychometric assessment. A score of ≥25% of skills not mastered in a domain is classified as delayed and demonstrates sound screening test characteristics (sensitivity 74%, specificity 83%) in children born preterm.

**Psychosocial and Neonatal Risks**

Psychosocial and neonatal risks were assessed by using the Brigance Observations of Psychosocial Risk Scale and those with ≥4 of 11 risk factors present were classified as having high psychosocial risk in accordance with the scoring manual. Assessments of neonatal factors were collected from patient records, and coded according to Australian and New Zealand Neonatal Network data definitions. This is a collaborative network of all Australian and New Zealand Neonatal Intensive Care Units that uses standardized data definitions for monitoring the care of high-risk newborn infants.

**Data Analysis**

Descriptive statistics were used to illustrate the prevalence of the ADOS-G and M-CHAT-FI classified ASD and the baseline, neonatal, and developmental characteristics by M-CHAT-FI screening status and the ADOS-G profile of the M-CHAT-FI positive screen children in the combined cohort. Univariable and multivariable analyses were used to examine which social, neonatal, and developmental factors were associated with the M-CHAT-FI. Analysis was adjusted for psychosocial risk, child gender, age, and profound disabled cases. The criterion for statistical significance for multivariable analysis was set at a 2-tailed value of .01 to control for family-wise error. Data are presented as means or percentages, odds ratios (ORs) with 95% confidence intervals (CIs), and P values for the combined cohort. Data were analyzed by using Statistical Package for Social Sciences for Windows (version 22.0; IBM SPSS Statistics, IBM Corporation, Chicago, IL).

**RESULTS**

Of the 192 surviving children, 169 (88%) participated in the study, 13 were lost to follow-up, and 10 parents refused their child’s participation. All participants completed the M-CHAT-FI and developmental follow-up assessments, 153 children completed the psychometric tests, and the remaining 16 children completed the PEDS:DM-AL (Fig 1). The baseline characteristics of the children in the study group (Table 1) and those not studied were comparable in terms of baseline, maternal, and neonatal characteristics.

**Prevalence of Screening ASD**

In total, 13% (22 of 169) of children screened positive for ASD by using the M-CHAT-FI. Nine (40.9%) of the 22 children failed ≥2 critical items, with the remainder failing any 3 items. Positive screening results (2 years 13.6% and 4 years 12%) and failing critical items (2 years, n = 4 of 11, 37%; and 4 years, n = 5 of 11, 45%) were similar for both age groups.

**Clinical Characteristics and Their Relationship to First-Stage Screen Results**

Univariable analyses are shown in Table 1. Multivariable analysis showed that only adaptive behavior was independently associated with M-CHAT-FI positive results (OR 15.0, 95% CI 5.7–43.4, P < .001), even after adjusting for psychosocial risk, gender, age, and profound disabled cases (OR 9.9, 95% CI 3.4–28.7, P < .001).
When we removed adaptive behavior from the multivariable analysis, language delay independently predicted the positive M-CHAT-FI screen (OR 4.3, 95% CI 1.5–14.7, *P* < .001). No pattern of distribution of positive M-CHAT-FI children by GA was identified.

### Prevalence of Diagnostic ASD

Of the 22 M-CHAT-FI positive screens eligible for ADOS-G assessment, 2 of the 2-year-old children lived remotely and were unable to return to the clinic; 1 of these had mild cerebral palsy and the other had a mild cognitive delay. Five children had severe-profound disability and could not be reliably assessed. Fifteen children (2 years, *n* = 7 and 4 years, *n* = 8) were assessed by using the ADOS-G. Four children were assessed using Module 1 and 11 were assessed using Module 2. The ADOS-G classified 1.8% (3 of 169) of cases with ASD with all cases meeting criteria for autism (the highest cutoff), which included two 2-year-olds and one 4-year-old child. No children were classified in the autism spectrum range (the lower cutoff score). Two children had

### TABLE 1 M-CHAT-FI Screening Status for ASD by Baseline, Neonatal, and Developmental Factors in 169 Children

<table>
<thead>
<tr>
<th>Factors</th>
<th>M-CHAT-FI Positive, <em>n</em> = 22</th>
<th>M-CHAT-FI Negative, <em>n</em> = 147</th>
<th>OR (95% CI)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA, wk, mean (±SD)</td>
<td>26.7 (1.4)</td>
<td>26.6 (1.4)</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>GA ≤ 26</td>
<td>8 (36)</td>
<td>68 (45)</td>
<td>0.7 (0.3–1.6)</td>
<td>.35</td>
</tr>
<tr>
<td>Boys</td>
<td>15 (68)</td>
<td>78 (53)</td>
<td>1.8 (0.7–4.8)</td>
<td>.20</td>
</tr>
<tr>
<td>Maternal age ≥ 35</td>
<td>4 (18)</td>
<td>27 (18)</td>
<td>1.0 (0.3–3.4)</td>
<td>.98</td>
</tr>
<tr>
<td>Plurality</td>
<td>4 (18)</td>
<td>46 (31)</td>
<td>0.5 (0.2–1.5)</td>
<td>.32</td>
</tr>
<tr>
<td>Psychosocial risk</td>
<td>5 (22)</td>
<td>19 (13)</td>
<td>1.9 (0.7–5.9)</td>
<td>.21</td>
</tr>
<tr>
<td><strong>Neonatal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinopathy of prematurity ≥ 3 or laser therapy</td>
<td>3 (13)</td>
<td>20 (13)</td>
<td>1.0 (0.3–3.7)</td>
<td>.97</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia, late-onset sepsis, or necrotizing enterocolitis</td>
<td>14 (63)</td>
<td>83 (56)</td>
<td>1.4 (0.5–3.4)</td>
<td>.53</td>
</tr>
<tr>
<td>Interventricular hemorrhage ≥ 3, cystic formation, or hydrocephalus</td>
<td>3 (13)</td>
<td>17 (11)</td>
<td>1.2 (0.3–4.5)</td>
<td>.72</td>
</tr>
<tr>
<td>No breastfeeding on discharge</td>
<td>10 (45)</td>
<td>34 (23)</td>
<td>2.8 (1.1–6.9)</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Development</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>8 (36)</td>
<td>15 (10)</td>
<td>5.0 (1.8–13.9)</td>
<td>.01</td>
</tr>
<tr>
<td>Vision or hearing impairment</td>
<td>2 (9)</td>
<td>3 (6)</td>
<td>3.4 (0.9–12.0)</td>
<td>.07</td>
</tr>
<tr>
<td>Cognitive delay &lt; –2 SD</td>
<td>11 (50)</td>
<td>19 (13)</td>
<td>6.7 (2.5–17.6)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Language delay &lt; –2 SD</td>
<td>12 (54)</td>
<td>23 (15)</td>
<td>6.4 (2.5–16.5)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Adaptive behavior delay &lt; –2 SD</td>
<td>15 (68)</td>
<td>20 (13)</td>
<td>13.8 (4.9–47.4)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Any atypical development</td>
<td>21 (95)</td>
<td>42 (28)</td>
<td>52.5 (6.8–400)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

All values are *n* (%) unless otherwise noted. OR indicates the OR for having a positive screen, given the presence of the factor. Atypical development indicates any cerebral palsy, blindness, or hearing impairment, or cognitive, language, or adaptive behavior delay.
significantly reduced imagination and play and 2 were observed to have some stereotypical behavior and restricted interest. All 3 children had a co-occurring neurodevelopmental problem (Supplemental Table 3). No misclassified cases were identified.

**ADOS-G Domain and Item Profiles of the Children Categorized Nonspectrum**

Eleven of the 12 children categorized as ADOS-G negative or nonspectrum scored some level of abnormality on ≥2 of the 4 domain categories on the ADOS-G assessment (Supplemental Table 3). One-third of these children reached the Communication domain lower cutoff threshold for ASD and had delayed cognition or language ability. Additionally, one-third of children had stereotypical behavior or restricted interests and more than one-third (5/12) showed reduced imaginative play or appropriate play with objects. All but 1 child experienced ≥1 co-occurring neurodevelopmental problems. The ADOS-G scores in each domain suggested a subthreshold social communication impairment in the ADOS-G negative children (Supplemental Table 3).

There were wide-ranging developmental delays in ADOS-G communication and social reciprocal interaction domain item scores in the M-CHAT-FI positive screen children. Most children who were ADOS-G negative did have mild communication impairment (item scores of 1), indicating these children were showing less of the communication behaviors than would be expected for their age (Table 2).

**DISCUSSION**

The current study sought to investigate the prevalence of ASD in toddlers born at <29 weeks’ GA by using a 2-stage procedure, within a developmental follow-up program, whereby positive M-CHAT-FI-screened children were diagnostically assessed by using the ADOS-G. Previous research using diagnostic assessment tools found an elevated risk of ASD across ages in children born preterm of between 3.6% and 12.9%, relative to term controls or general population prevalence <1%.3,4,7 The diagnostic prevalence in the current study of 1.8% was composed exclusively of children meeting the cutoff for autism. However, compared with the general population, the elevated prevalence of the more narrowly...
defined autism was consistent with previous studies of ASD in the preterm population in both toddlers and older children. Our lower prevalence may have been an effect of the ADOS-G, which is less sensitive to lower compared with higher threshold scores.

The only other diagnostic study in toddlers is by Dudova et al who used the ADOS-G, and who found prevalence in 2-year-old children with birth weight <1500 g and without major disability that was 7 times greater than our prevalence. Potential explanations for this discrepancy are the high loss to follow-up (>40%) and potential rating bias in the previous study. Furthermore, the Dudova et al study used birth weight rather than GA as the inclusion criteria and relied on clinical judgment of cognitive and motor development, which may have led to important differences in the sample under investigation. Unlike our study, Dudova applied a concept of best-estimate clinical diagnosis, by consensus of 2 experienced specialists, and reported 1 misclassified ADOS-G–positive case. In our study, we reviewed all screening and diagnostic cases in the overall context of their developmental assessments and found no misclassified cases. As we found no autism spectrum cases (lower threshold scores), it is possible that we missed higher-functioning ASD cases. The study by Pinto-Martin et al used the ADOS-G and Autism Diagnostic Interview-Revised and found 2.5% of nonspectrum adults met the ADOS-G criteria at the lower-threshold autism spectrum and had higher intelligence scores compared with the screen-positive cases. It is likely that the method of determining misclassified cases will affect estimates. Further research investigating all cases and the links between developmental functioning, birth weight, GA, and ASD is needed to elucidate the mechanism or mechanisms that lead to an elevated rate and classification differences within ASD in children born preterm.

Previous research with the preterm population also has reported high positive screening rates for ASD. The M-CHAT is the most frequent ASD screen reported in the preterm toddler population and has consistently yielded high screening rates (21%–41%) even when adjusted for disability (10%–16.5%). With the introduction of the FI for positive M-CHAT screens, the rate in both late and very preterm toddlers has recently been reported at 2.4% and 3.1%, respectively. Our rate of 13%, although predictably lower than those reports using the M-CHAT without the FI, was more than 4 times higher than that reported in studies using the FI. Our rate of positive screens adjusted for co-occurring disability was 3.6% and similar to that reported by the studies using the M-CHAT-FI. We may have been less restrictive in our use of the FI, and although we report a higher screening rate, we also describe the false-positive screens with subthreshold communication impairment. We anticipate that some of these children may be later diagnosed with ASD.

It is unclear if the use of multiple screening tests improves accurate identification of ASD in the preterm population. In older-aged cohorts, the use of multiple screens yields similar screening rates between 18.3% and 15.8% and diagnostic prevalence rates between 5% and 8%. In toddlers born preterm, the use of multiple screens has yielded high but varied positive rates (20%–35.7% on any screen; 1%–9% on all screens). Only 1 study, by Dudova et al, used diagnostic validation for combinations of screens and demonstrated sensitivity and specificity >76% in all combinations except when all 3 screen tests had to be positive, which yielded a sensitivity of 23%.

It is not clear how the associated impaired development in children born preterm contributes to the development of ASD. Like other studies, we demonstrated a significantly increased rate of cerebral palsy and cognition and language delay in the M-CHAT-FI–positive toddlers. Language delay was the only delay that predicted positive ASD screens; however, when we included adaptive behavior, it showed the strongest predictor of screen-positive cases. This is not surprising, as communication and social skills deficits are both characteristics of ASD and pivotal aspects of adaptive behavior. Importantly though, a subthreshold social communication profile was very common in the ADOS-G nonspectrum children who screened M-CHAT-FI positive. This profile has been shown repeatedly in other studies suggesting false-positive children may meet the criteria for the new category of Social Communication Disorder by using Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria.

Developmental surveillance of this subthreshold group may also be of assistance in monitoring whether these children later meet diagnostic criteria as further symptoms manifest when social demands exceed capacities. Continued surveillance of this cohort is ongoing and recently conducted longitudinal assessment may reveal additional cases.

The current study was limited through using the ADOS-G Module 1 or 2. Since the time of data collection for these studies, a revised form that
includes a toddler module has been developed that may be more sensitive to this age and ability range.\textsuperscript{41} Furthermore, the absence of control cases may limit comparisons to other groups. Emerging research suggests a developmental surveillance approach that begins earlier in infancy may be more sensitive to identification of ASD in young children.\textsuperscript{42} Testing the applicability of this approach to children born preterm would be an important area for further research. Nevertheless, the current 2-stage approach is likely to be a feasible approach to identifying young children at risk for ASD in clinical practice.

**CONCLUSIONS**

This study provides further support of elevated rates of autistic disorder and an associated subthreshold social communication profile in the preterm population, although our data suggest rates may not be as high as previously reported. Further research is needed to understand the mechanisms leading to an elevated prevalence of ASD in the preterm population. The current study highlights the need for assessment of ASD in children born preterm because of the heightened risk that symptoms may be overlooked due to the range of other developmental problems.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADOS-G</td>
<td>Autism Diagnostic Observation Schedule-Generic</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism spectrum disorder</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>GA</td>
<td>gestational age</td>
</tr>
<tr>
<td>M-CHAT-FI</td>
<td>Modified Checklist of Autism in Toddlers—Follow-up Interview</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>Peds:DM-A</td>
<td>Parents’ Evaluation of Developmental Status: Developmental Milestones–Assessment Level</td>
</tr>
</tbody>
</table>

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**Potential Conflict of Interest:** The authors have indicated they have no potential conflicts of interest to disclose.

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26. Wechsler D. Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III Australia)

Autism in Toddlers Born Very Preterm

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