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## Title page

### Clinical Features of Children with Autism Who Passed 18-Month Screening

Roald A. Øien<sup>1,2</sup>, MA, Synnve Schjølberg<sup>3</sup>, Cand.psych., Fred R. Volkmar<sup>2</sup>, MD, Frederick Shic<sup>4,5</sup>, PhD, Domenic V. Cicchetti<sup>2</sup>, PhD, Anders Nordahl-Hansen<sup>6</sup>, PhD, Nina Stenberg<sup>7</sup>, PhD, Mady Hornig<sup>8,12</sup>, MD, Alexandra Havdahl<sup>9,3</sup>, PhD, Anne-Siri Øyen<sup>10,3</sup>, PhD, Pamela Ventola<sup>2</sup>, PhD, Ezra S. Susser<sup>8,11</sup>, MD, Martin R. Eisemann<sup>1</sup>, PhD, Katarzyna Chawarska<sup>2</sup>, PhD.

#### **Affiliations:**

1. Department of Psychology, UiT – The Arctic University of Norway, Tromsø, Norway
2. Child Study Center, School of Medicine, Yale University, New Haven, USA
3. Division of Mental and Physical Health, Norwegian Institute of Public Health, Oslo, Norway
4. Center for Child Health, Behavior and Development, Seattle Children's Research Institute, Seattle, WA, USA
5. Department of Pediatrics, University of Washington, Seattle, WA, USA
6. Department of Special Needs Education, University of Oslo, Norway
7. Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway
8. Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, USA
9. MRC Integrative Epidemiology Unit, Bristol Medical School, University of Bristol, Bristol, UK
10. Nic Waals Institute, Lovisenberg Hospital, Oslo, Norway
11. New York State Psychiatric Institute, NY, USA
12. Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, USA

**Address correspondence to:** Roald A. Øien, Department of Psychology, UiT – The Arctic University of Norway. 9037 Tromsø, Norway. [roald.a.oien@uit.no;roald.oien@yale.edu] +47 77644344/+47 93099994(cell)

**Short title:** Children with Autism Who Passed 18-Month Screening

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PCT/US2013/028589, and WO2010147714A1). No funding has been received from these patents at the time of the writing of this manuscript. The other authors have indicated that they have no potential conflicts of interest to disclose.

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

**Abbreviations:** PPV – Positive Predictive Value, NPV – Negative Predictive Value, SE – Sensitivity, SP – Specificity, ASD – Autism spectrum disorder, M-CHAT – Modified Checklist for Autism in Toddlers, ASQ – Ages and Stages questionnaire, EAS – Early Temperament Survey, NPR – Norwegian Patient Registry, IQ – Intelligence Quotient, MoBa – Norwegian Mother and Child Study

### **Table of Contents Summary**

This is the first study to examine developmental and temperamental characteristics of boys and girls screening negative for autism at 18 months.

### **What's Known on This Subject**

To the authors' best knowledge, no study has examined the clinical characteristics of children who pass screening for ASD at 18 months but are later diagnosed with the disorder.

### **What This Study Adds**

The present study suggests that despite passing screening for ASD, 18-month-old males and females later diagnosed with ASD show delays and atypical features in social, communication, and motor domains at the time of the screening.

### **Contributors' Statement:**

Mr. Øien, Mrs. Schjøberg, Drs. Volkmar, Shic & Chawarska conceptualized and designed the study, conducted the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. Drs. Havdahl, Nordahl-Hansen, Hornig, Stenberg, Øyen, Ventola, Susser and Eisemann edited and critically reviewed the manuscript and its analyses. Dr. Cicchetti supervised and critically reviewed the analyses and reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

## Abstract

**Objectives:** We compared sex-stratified developmental and temperamental profiles at 18 months in children screening negative for autism spectrum disorder (ASD) on the Modified Checklist for Autism in Toddlers (M-CHAT), but later receiving diagnoses of ASD (False(-) group), vs. those without later ASD diagnoses (True(-) group).

**Methods:** 68,197 screen-negative cases from the Norwegian Mother and Child Cohort (MoBa) were included (49.1% females). Children were screened using the six critical items of the M-CHAT at 18 months. Groups were compared on domains of the Ages and Stages Questionnaire and the Emotionality, Activity and Sociability Temperament Survey.

**Results:** Despite passing M-CHAT screening at 18 months, children in the False(-) group exhibited delays in social, communication, and motor skills compared to the True(-) group. Differences were more pronounced in females. However, with regard to shyness, males in the False(-) group were rated as more shy than their True(-) counterparts, but females in the False(-) group were rated as less shy than their counterparts in the True(-) group.

**Conclusion:** This is the first study to indicate that children who pass M-CHAT screening at 18 months, and later diagnosed with ASD, exhibit delays in core social and communication areas as well as fine motor skills at 18 months. The differences appeared to be more pronounced in females. The findings underscore a need to enhance the understanding of early markers of ASD in males and females, as well as factors affecting parental report on early delays and abnormalities, in service of improving sensitivity of early screening instruments.

## Introduction

The primary goal of ASD screening instruments is to facilitate early identification and implementation of early interventions. However, as most studies are conducted in clinical populations, it is unclear if existing screening instruments have sufficiently high sensitivity (Se), specificity (Sp), and positive predictive value (PPV) in general population-based samples.<sup>1,2</sup> Further, there is increasing awareness of substantial heterogeneity with respect to both timing of the onset of recognizable symptoms<sup>3</sup> and patterns of symptom expression.<sup>4</sup> Recognizing that symptoms of ASD may become apparent at different ages as social demands begin to exceed a child's limitations, the strict age-of-onset criterion in previous formal definitions of ASD has been removed from the DSM-5.<sup>5</sup> Moreover, recent prospective studies of infants at familial risk for ASD suggest that symptoms of ASD may manifest somewhat differently depending on a child's verbal and nonverbal levels of functioning.<sup>6</sup>

The Modified Checklist for Autism in Toddlers (M-CHAT)<sup>7</sup> is the most widely used screening instrument for ASD in young children.<sup>8</sup> Designed to be completed in the waiting room of a primary care provider,<sup>7</sup> it has been recommended for use in toddlers at 18 months of age with a follow-up at 24 months.<sup>9</sup> Although studies of the M-CHAT typically demonstrate its high sensitivity in clinical samples, it has been criticized for its lower specificity and PPV. In an unselected population sample, Stenberg and colleagues (2014)<sup>10</sup> reported a PPV of 3.3% using the M-CHAT's six-critical item criterion and 1.5% using the total 23-item criterion in a general population sample. In selected populations, i.e., children with developmental concerns, the M-CHAT performs better at detecting children at risk of ASD.<sup>7,11,12</sup> A critical gap in the current evidence stems from lack of prospective follow-up studies of children who screen negative.<sup>1</sup>

To the best of our knowledge, no study has yet investigated the developmental and temperamental characteristics of children who are screen-negative based on M-CHAT at 18

months of age, but later receive ASD diagnosis. Understanding how early symptoms manifest in this group of children is of paramount significance for development of future ASD specific screening instruments. There are multiple reasons why a child with ASD may pass early screening only to be diagnosed with ASD later in childhood, apart from simply experiencing later symptom onset. Limited parental knowledge or understanding of the screening questions may also be an issue, though recent studies have found good agreement between parents and clinicians on ratings of autism-related behaviors amongst parents of infants at risk for ASD.<sup>13,14</sup> Studies also point that child related factors such as better developed language<sup>15</sup> and absence of repetitive and restricted behaviors, average-range IQ, younger age at assessment<sup>16,17</sup> and lack of additional behavioral issues,<sup>18</sup> which may mask symptoms of social disability. Incorporating other measures that more broadly examine developmental features and consider children's developmental level could provide new insights with regard to earlier identification of children with ASD. Moreover, given multiple reports suggesting sex differences in syndrome expression,<sup>19-23</sup> there is great need to evaluate performance of existing screeners in both males and females.

The present study examined developmental and temperamental characteristics of children who passed the six-critical item criterion of the M-CHAT at 18 months but went on to receive an ASD diagnosis. Specifically, we compared screen-negative children without a later ASD diagnosis (True(-) group) to screen-negative children with a later ASD diagnosis (False(-) group) on a set of developmental and temperamental features also measured at age 18 months. The study capitalized on data collected through the Norwegian Mother and Child study (MoBa),<sup>24</sup> a prospective, country-wide pregnancy cohort of parents recruited at the 18<sup>th</sup>-gestational week ultrasound examination and followed regularly with questionnaires related to child development. The M-CHAT,<sup>7</sup> along with other developmental scales, was part of the 18-month MoBa questionnaire. Examination of characteristics in screen-negative children

may facilitate identification of new behavioral markers of ASD at critical time points for emergence of frank behavioral symptoms of ASD.

## **Methods**

### **Study population**

The study sample is derived from the Norwegian MoBa Study.<sup>24</sup> In total, 40.6% of invited mothers consented to participate. Diagnoses of ASD were obtained from the Autism Birth Cohort (ABC), a sub-study in MoBa<sup>25</sup> that integrates diagnoses from ABC Clinic assessments at child age 40 months and older and diagnoses obtained through annual linkage with the Norwegian Patient Registry (NPR). NPR is a national database of all discharge diagnoses of patients assessed in health care services across Norway. It has been available since 2008. According to national guidelines at specialist health care in Norway, the use of ADOS and ADI-R in the diagnostic process is mandatory, together with a range of other tests/interviews on cognitive and adaptive function. The MoBa and the ABC study obtained written informed consent from participating mothers and were approved by the Norwegian Data Inspectorate, as well as the Regional Committee for Medical and Health Research Ethics South-East Norway (REK). The present study used the MoBa data release version 9, reflecting diagnoses collected throughout 2015.

----INSERT FIGURE 1 ABOUT HERE----

### **Measures**

MoBa questionnaires completed when the child was 18 months old included the M-CHAT, selected items from the Ages and Stages Questionnaire (ASQ)<sup>26</sup> and the Emotionality, Activity and Sociability Temperament Survey (EAS).<sup>27</sup> The M-CHAT is a 23-item screening instrument<sup>7</sup> with each item scored either as pass or fail. Six out of the 23 items are considered



critical for predicting an ASD diagnosis,<sup>7</sup> as the items probe for social and communicative behaviors such as pointing, interest in other children, imitation, and response to his/her own name (Appendix 1a).

Children are considered screen-positive if they fail 2 or more of the 6 critical items. For the purpose of this study, individual scores on the six critical items were summarized and children receiving scores less than 2 were categorized as screen-negative. The focus on the six critical items was motivated by findings that this criterion provides the best precision for predicting ASD.<sup>7,10,11,28</sup> The means of the six critical items are listed in Table 1.

Ages and Stages Questionnaire (ASQ) is a parent-reported questionnaire designed to measure developmental skills from age 4 months to 5 years.<sup>26</sup> For each item, parents are asked to rate whether specific behaviors are currently present: “yes” (10), present “sometimes” (5) and “not yet” present (0). Thus, a higher score indicates more normative development. A subset of 13 items falling into four ASQ-defined domains (Social, Communication, Fine Motor and Gross Motor) were included in the MoBa 18-month questionnaire (Appendix 1b).

Emotionality, Activity and Sociability Temperament Survey (EAS)<sup>27</sup> was designed for children aged 1 to 9 years and measures emotionality, activity, sociability and shyness. For each item, the parent is asked to rate her/his child on a 5-point rating scale (from 1: very characteristic/typical of your child, to 5: not characteristic/typical of your child). A subset of 11 items of the EAS<sup>29</sup> falling into four EAS-defined domains (Sociability, Shyness, Activity and Emotionality) were included in the MoBa 18-month questionnaire (Appendix 1c). Items were coded such that a higher score on all domains indicated more sociable and active traits, and less shy and emotional traits.

----INSERT TABLE 1 ABOUT HERE----

## Statistical Analyses

To compare children in the True(-) group to children in the False(-) group, we conducted a set of univariate ANOVAs with diagnosis (ASD, no ASD) and sex (male, female) as between-group factors on the ASQ and EAS domain scores. Post-hoc analyses were conducted for between- and within-group differences, utilizing independent samples. Analyses comparing True(+) to to False(-) are attached (Appendix 3). Bonferroni correction was used to control for multiple comparisons, and Cohen's *d* provided a measure of effect sizes in the independent-samples analyses. Cohen's *d* was interpreted as follows: T = Trivial, S = Small, M = Moderate, L = Large.<sup>30</sup>

## Results

Of 69,668 children with all six-critical items completed at 18-month screening, 1,471 screened positive and 68,197 screened negative. Among those screening negative, 49.1% were females. Of the 68,197 screen-negative children, 228 (15.8% males) were later diagnosed with ASD (False(-) children).

INSERT FIGURE 2A and 2B here (alongside)

### Developmental domains (ASQ)

----INSERT TABLE 2a ABOUT HERE----

Social domain. Analyses indicated a significant effect of diagnosis ( $p < 0.001$ ), no effect of sex ( $p = 0.551$ ), and a significant diagnosis-by-sex interaction ( $p = 0.001$ ). Males in the False(-) group were rated as less social than True(-) males ( $p < 0.001$ ,  $d = 0.303$ [S]). Females in the False(-) group were also rated as with fewer social skills than True(-) females, ( $p = 0.007$ ,  $d = 0.657$ [M]), but the magnitude of the difference was larger than that observed in males. No significant differences were found between male and females in the False(-) group ( $p = 0.329$ ,  $d = 0.203$ [S]). However, True(-) females had higher scores on social skills than True(-) males ( $p < 0.001$ ,  $d = 0.255$ [S]).

Communication domain. Analyses indicated a significant effect of diagnosis ( $p < 0.001$ ), no effect of sex ( $p = 0.366$ ), and a diagnosis-by-sex interaction ( $p = 0.002$ ). There was a difference between the False(-) and True(-) males in communication skills ( $p < 0.001$ ,  $d = 0.608[M]$ ), as well as between the False(-) and True(-) females ( $p < 0.001$ ,  $d = 1.13[L]$ ). The magnitude of the effect was greater in females. No differences were found between males and females in the False(-) groups ( $p = 0.414$ ,  $d = 0.152[T]$ ), but True(-) females scored higher than True(-) males ( $p < 0.001$ ,  $d = 0.380[S]$ ).

Fine motor domain. Analyses revealed significant effects of diagnosis ( $p < 0.001$ ) and sex ( $p = 0.017$ ), but no interaction between the factors ( $p = 0.152$ ). Children in the False(-) group had, in general, less developed fine motor skills than children in the True(-) group ( $p < 0.001$ ,  $d = 0.399[S]$ ). Females were generally less advanced in fine motor skills than males ( $p < 0.001$ ,  $d = 0.088[T]$ ), regardless of diagnosis.

Gross motor domain. Analyses indicated significant effects of diagnosis ( $p < 0.001$ ) and sex ( $p < 0.001$ ), and a diagnosis-by-sex interaction ( $p < 0.001$ ). There was a differences between the False(-) and True(-) males in gross motor skills ( $p < 0.001$ ,  $d = 0.267[S]$ ), as well as between the False(-) and True(-) females ( $p < 0.001$ ,  $d = 1.06[L]$ ). The magnitude of the effect was greater in females. Females in the False(-) group had lower scores than males in the False(-) group ( $p = 0.001$ ,  $d = 0.779[M]$ ), and True(-) females had lower scores than True(-) males ( $p = 0.005$ ,  $d = 0.022[T]$ ).

INSERT FIGURE 2C and 2D here (alongside)

### **Temperamental characteristics (EAS subdomains)**

----INSERT TABLE 2b ABOUT HERE----

Sociability: Analyses indicated a significant effect of diagnosis ( $p < 0.001$ ), no effect of sex ( $p = 0.156$ ), and no interaction effect ( $p = 0.260$ ). Post-hoc analyses indicated that children

in the False(-) group were rated as less sociable than children in the True(-) group, regardless of their sex ( $p < 0.001$ ,  $d = 0.403[S]$ ).

Shyness: Analyses indicated no effects of diagnosis ( $p = 0.551$ ) or sex ( $p = 0.060$ ), but a significant diagnosis-by-sex interaction ( $p = 0.001$ ). Post-hoc analyses indicated that males in the False(-) group were rated as more shy than males in the True(-) group ( $p = 0.003$ ,  $d = 0.238[S]$ ). Females in the False(-) group were rated as less shy than females in the True(-) group ( $p = 0.035$ ,  $d = 0.369[S]$ ). Females in the False(-) group were rated as less shy than males in the False(-) group ( $p = 0.017$ ,  $d = 0.463[S]$ ). Furthermore, females in the True(-) were rated as more shy than males in the True(-) group ( $p < 0.001$ ,  $d = 0.134[T]$ ).

Emotionality: Analyses indicated no significant effects of diagnosis ( $p = 0.069$ ), sex ( $p = 0.607$ ), or interaction between diagnosis and sex ( $p = 0.435$ ).

Activity: Analyses indicated significant effects of diagnosis ( $p = 0.036$ ) and sex ( $p < 0.001$ ), but no interaction effects ( $p = 0.114$ ). Post-hoc analyses showed no difference between children in the False(-) and True(-) groups ( $p = 0.664$ ). Females were in general less active than males ( $p < 0.001$ ,  $d = 0.183[T]$ ), regardless of diagnosis.

## Discussion

To the best of our knowledge, this study is the first to investigate the concurrent developmental and temperamental characteristics of males and females who pass the six-critical item criterion of the M-CHAT at 18 months of age, but ultimately receive ASD diagnosis at a later age. Utilizing a large prospective population study, we compared false screen-negative children to true screen-negative children on their characteristics as measured concurrently with M-CHAT screening at 18 months.

Despite screening negative for ASD on the M-CHAT, children in the False(-) group exhibited delays and atypical features compared to children in the True(-) group. Specifically, children in the False(-) group were already rated by their parents at 18 months as having less

developed social and communication skills as well as showing fine and gross motor delays compared to children in the True(-) group. The domains of impairment identified in the current study map onto those found in children with autism diagnosed in the second year of life,<sup>6,31</sup> suggesting that atypical features in the false negative cases may already be present at 18 months. There were no marked differences between males and females as in most cases, both males and females in the False(-) group performed more poorly than their sex-matched counterparts in the True(-) group. However, the observed differences, as indexed by effect sizes, appeared more pronounced in females, particularly in social, communication, and gross motor domains. There was only one area where males and females showed a different pattern: males in the False(-) group were rated as more shy than males in the True(-) group, whereas females in the False(-) group were rated as less shy than females in the True(-) group. These findings suggest that already at 18 months there are nuanced differences in temperamental indices between males and females who screen negative and later receive an ASD diagnosis.

Intriguingly, females in the False(-) group were rated as less socially inhibited compared to males. This is in contrast to the pattern found in the True(-) group. A closer inspection of the Shyness domain revealed that females in the False(-) group had shorter warm-up time and appeared friendlier toward strangers than males in the False(-) group (Appendix 2). We hypothesize that females in the False(-) group have somewhat lower levels of social fearfulness or lower inhibitory control compared to males. Studies have revealed that in typically-developing children, females show greater inhibitory control compared to males.<sup>32</sup> The sparse research on inhibitory control in individuals with ASD also suggests that females with ASD express less inhibition,<sup>33,34</sup> and the lack of knowledge about sex differences in fearfulness amongst young children with ASD. Future studies should examine the levels of social fearfulness and inhibitory control during infancy and early childhood in ASD, as these processes have a great capacity to shape the emerging autism phenotypes and contribute to the heterogeneity in syndrome expression.

The results also revealed sex differences that were independent of the ASD outcome. Specifically, males in both groups were more advanced than females in gross motor skills, a finding consistent with earlier work in children with ASD<sup>35,36</sup> as well as in typically-developing children.<sup>37,38</sup> Furthermore, consistent with prior work,<sup>21,39</sup> males had a higher activity level than females.

The present study revealed that despite passing the M-CHAT six-critical item criterion, 18-month-old False(-) children show atypical features compared to children in the True(-) group. Importantly, all instruments considered in the present study were completed by parents around the same age of the child, thus, recall bias and hindsight are unlikely to explain these disparities. At present, it is not clear what contributed to the observed differences between instruments, but several hypotheses can be advanced. First, parents may have difficulties mapping specific behavioral markers considered in the M-CHAT onto their children's real-life behaviors. They may also have difficulties understanding some of the phenomenology of more specific or rare behaviors related to ASD. Moreover, M-CHAT items do not provide opportunities for graded responses, which might affect how parents weigh their answer. The ASQ gives parents the opportunity to express that the child exhibits skills occasionally albeit inconsistently, which may allow them to express their concerns and perceptions in a more graded manner. Finally, it is also likely that symptoms of ASD may be expressed differently in early childhood, depending on the child's specific level of verbal and nonverbal skills,<sup>6</sup> or temperamental characteristics. A study in a large sample of infants at risk for ASD suggests that at 18 months children who display more prototypical symptoms of ASD tend to have lower verbal and nonverbal skills than those who are later diagnosed with ASD but show presentation at 18 months is less typical.<sup>6</sup> To date, few ASD specific screening instrument provides accommodations or modifications for variation in language level, though direct diagnostic measures such as the ADOS-2 considers verbal level when selecting the algorithm items that are most likely to identify children with ASD.<sup>40</sup> There are,

however, ongoing efforts to develop autism screeners sensitive to chronological age.<sup>41,42</sup>

Similarly, future studies should examine directly the effects of cognitive and temperament variables on early phenotypic expression of ASD and evaluate if taking these under consideration may improve early detection.

It should be noted that the M-CHAT<sup>7</sup> screener used in the present study has undergone recent revisions, leading to the introduction of the M-CHAT R/F<sup>12</sup> aimed at decreasing screen-positives while retaining sensitivity. The number of questions in the M-CHAT was decreased by three, and the six-critical item criterion was abandoned such that the M-CHAT R/F now consists of 20 items and has new cut-offs and a recommended follow-up interview to provide greater utility at the diagnostic margins. The newly proposed M-CHAT-R/F cut offs suggest improved ASD detection and diminished rates of false positives. However, given lack of a comprehensive prospective follow up of screen-negative cases, it is not clear whether these changes also led to decreased false negative rates. Considering that population-based studies that focus on screening for developmental disorders and that incorporate long-term follow-up of all recruits are rare and take a long time to complete, it may be some time before the M-CHAT R/F will be scrutinized in a similar fashion as the original M-CHAT in this present study.

We believe that our results contribute, at a fundamental level, to our understanding of early screening for ASD, highlighting the discrepancy between hard cut-off criteria for autism and the social-communicative, developmental, and temperamental signatures of emerging or sub-threshold autism phenotypes. This issue will likely be universal to all parent-directed screening efforts for the foreseeable future. Further research utilizing measures that incorporate levels of verbal and nonverbal skills<sup>6</sup> and temperamental characteristics may prove useful for the development of screening instruments with an improved capacity for identifying children on the autism spectrum in the second year of life. There is also a need to optimize screener design and delivery to fully capitalize on parental knowledge of their

child. The results also reveal a unique quality of girls who screen negative but who are later diagnosed with ASD, namely diminished shyness or social inhibition. Given that these dimensions are not captured by either the M-CHAT or M-CHAT R/F, this novel finding adds critical knowledge to our understanding of the role of sex in shaping early autistic phenotypes and highlights the importance of considering sex differences in early screening and diagnosis. The study also expands the state-of-art pediatric practice by emphasizing that when trying to determine if a young child is exhibiting autism symptoms, clinicians should not rely solely on a single instrument, but consider parental concerns and draw upon other developmental surveillance instruments as well as their clinical judgment. The clinicians also need to be particularly wary about discounting symptoms of social difficulties in females, as they maybe masked by limited shyness or social inhibition.

### **Strengths & Limitations**

Limitations of the current study include the lack of concurrent direct measures of verbal and nonverbal developmental levels and absence of data regarding the severity of autism symptoms (e.g. ADOS-2 or ADI-R). Furthermore, the measures used in the present investigation were restricted to subsets of items from the ASQ and EAS, making it difficult to utilize cut-offs for clinical concern. Future replication studies should strive to include full-scale measures. The strengths of the study include the prospective design of the MoBa, the data from an unselected general population, and the ability to examine outcomes of screen negatives across time by identifying ASD children at later time points through the NPR. Future prospective population studies should also conduct screening at 24 months of age, according to American Academy of Pediatrics' guidelines on screening.



## Conclusions

This is the first study to indicate that despite passing the autism-specific screening at 18 months, both males and females who later receive a diagnosis of ASD show delays and atypical features in social, communication, and motor domains. This information was collected via parent report concurrently to the autism-specific screening. These findings suggest that there is a pressing need for enhancing our understanding on how to improve screening instruments, including evaluation of how well the intended meaning of items is understood and interpreted by parents and how patterns of atypical behavior stratify developmentally by sex. Key future questions involve whether the range of response options provided for each item is sufficiently granular, and if new or adapted screening items might improve capture of the early symptom profiles found here or identify characteristics of lower- and higher-functioning subsets of children. To maximize opportunities for early ascertainment of the broader range of children who will ultimately receive an ASD diagnosis, screening instruments should be refined to improve capacity for identifying the patterns of deficits that appear to emerge in early life among these later-diagnosed children who escape detection by current screening algorithms.

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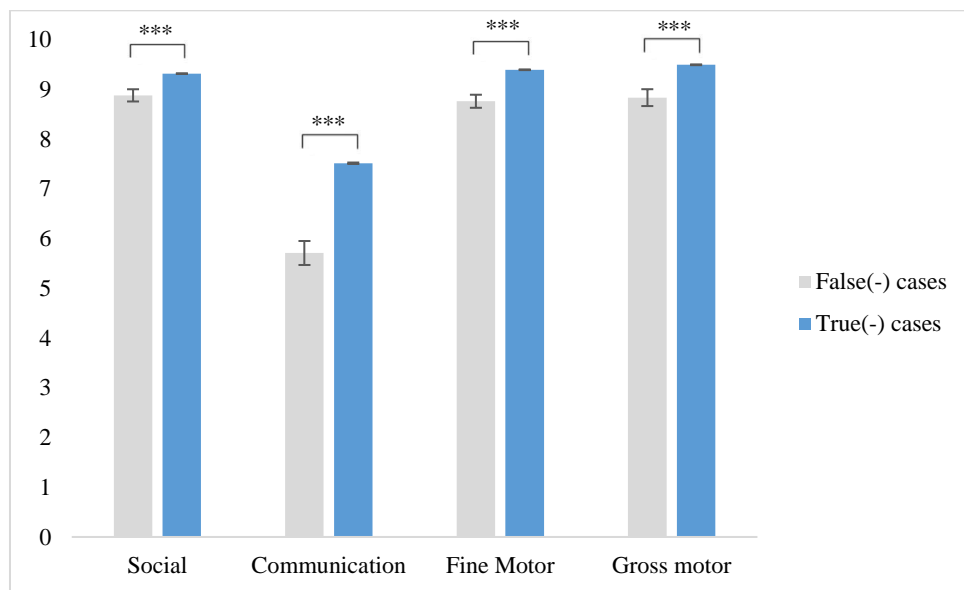
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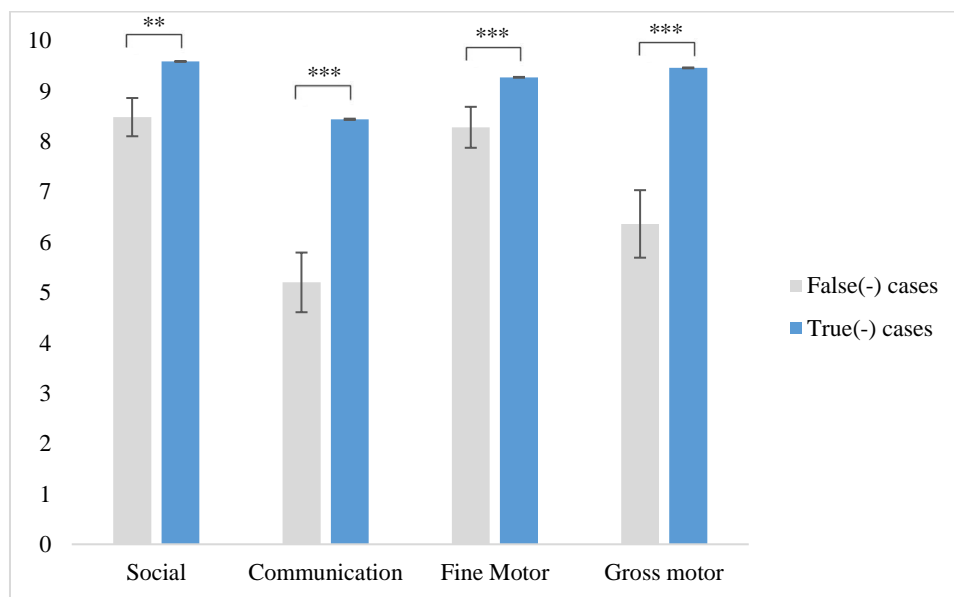
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**Figure 2a** Mean ( $\pm$  1 standard error) of the ASQ scores for False(-) and True(-) males.

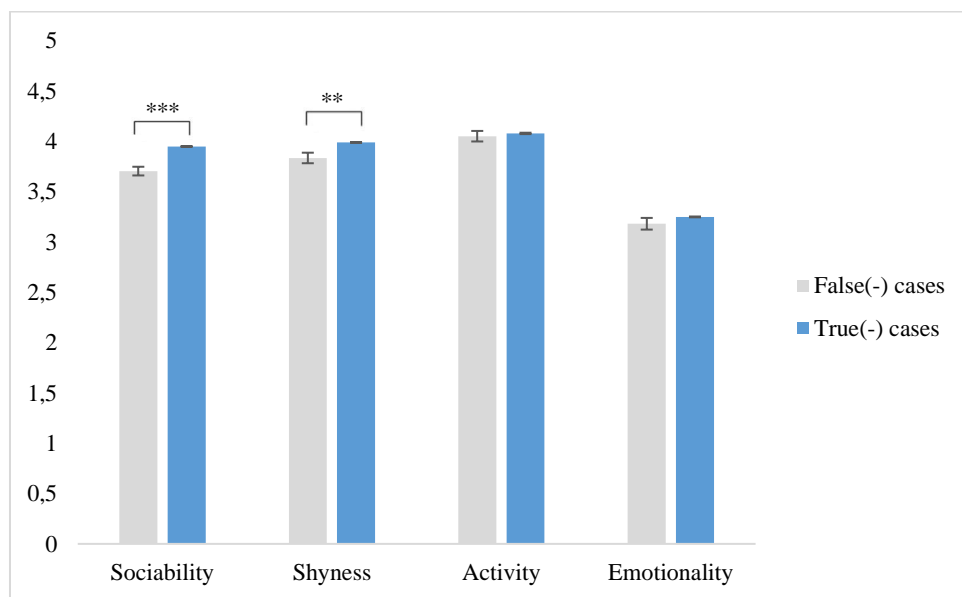


\*\*\*  $p < .001$

**Figure 2b** Mean ( $\pm$  1 standard error) of the ASQ scores for False(-) and True(-) females.

\*\*\*  $p < .001$  \*\*  $p < .010$

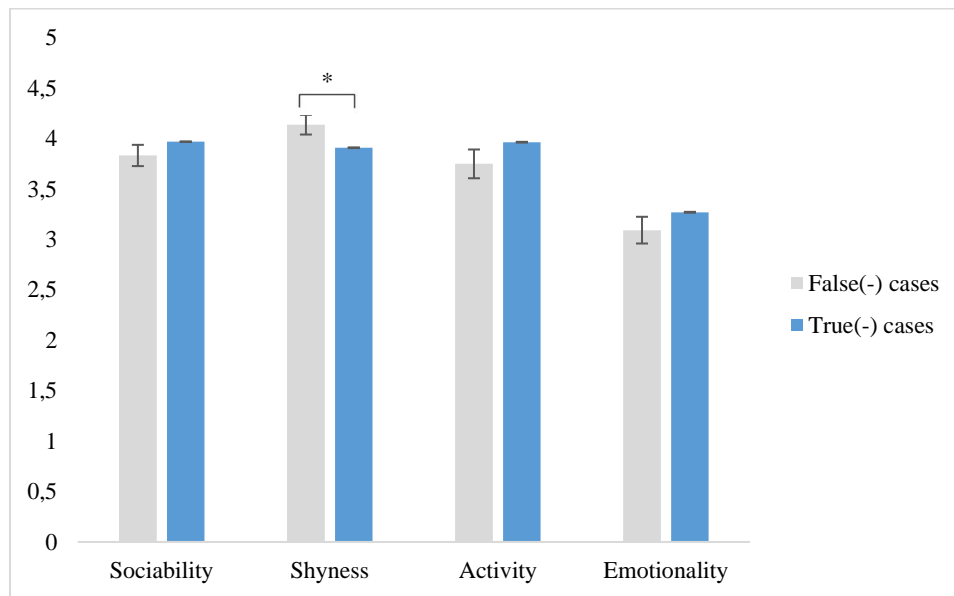
**Figure 2c** Mean ( $\pm$  1 standard error) of the EAS scores for False(-) and True(-) males.



\*\*\*  $p < .001$  \*\*  $p < .010$  - Higher score on Shyness and Emotionality scales indicate less shy and emotional presentation, higher Sociability and Activity scores indicate more pronounced characteristics in this domain



**Figure 2d** Mean (+/- 1 standard error) of the EAS scores for False(-) and True(-) females.



\*  $p < .050$  - Higher score on Shyness and Emotionality scales indicate less shy and emotional presentation, higher Sociability and Activity scores indicate more pronounced characteristics in this domain

**Table 1** Number of cases, Mean (SD) of participants age at time of screening and of failed M-CHAT six critical items

	Total True(-)	Males True(-)	Females True(-)	Total False(-)	Males (False-)	Females with (False-)
Number of cases	67,969	34,502	33,467	228	192	36
Age at time of screening (months)	18.53 (0.62)	18.53 (0.64)	18.53 (0.60)	18.51 (0.55)	18.51 (0.56)	18.53 (0.48)
Failed M-CHAT six critical items	0.10 (0.30)	0.12 (0.32)	0.08 (0.28)	0.27 (0.44)	0.25 (0.43)	0.41 (0.50)

**Table 2a** Mean (SD) of the Ages and Stages Questionnaire scores for males and females in the True and False Negative groups

	N	ASQ Social	ASQ Communication	ASQ Fine Motor	ASQ Gross Motor
Males – True(-)	33 163	9.32 (1.18)	7.51 (2.64)	9.39 (1.27)	9.49 (1.40)
Females – True(-)	32 231	9.59 (0.94)	8.44 (2.21)	9.28 (1.37)	9.46 (1.49)
Males – False(-)	183	8.88 (1.66)	5.71 (3.26)	8.76 (1.78)	8.83 (2.29)
Females – False(-)	33	8.48 (2.18)	5.20 (3.40)	8.28 (2.34)	6.36 (3.85)

**Table 2b** Mean (SD) of the Emotionality, Activity and Sociability Temperament Survey scores for males and females in the True and False Negative groups.

	N	EAS Sociability	EAS Shyness	EAS Emotionality	EAS Activity
Males – True(-)	33 300	3.95 (0.55)	3.99 (0.62)	3.25 (0.75)	4.08 (0.64)
Females – True(-)	32 347	3.96 (0.55)	3.91 (0.65)	3.27 (0.76)	3.96 (0.64)
Males – False(-)	185	3.96 (0.59)	3.84 (0.71)	3.18 (0.79)	4.05 (0.71)
Females – False(-)	36	3.83 (0.63)	4.14 (0.59)	3.09 (0.79)	3.75 (0.86)

Higher score on Shyness and Emotionality scales indicate less shy and emotional presentation, higher Sociability and Activity scores indicate more pronounced characteristics in this domain