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Eye Tracking Reveals Abnormal Visual Preference for Geometric Images as an Early Biomarker of an Autism Spectrum Disorder Subtype Associated With Increased Symptom Severity

Karen Pierce, Steven Marinero, Roxana Hazin, Benjamin McKenna, Cynthia Carter Barnes, and Ajith Malige

ABSTRACT

BACKGROUND: Clinically and biologically, autism spectrum disorder (ASD) is heterogeneous. Unusual patterns of visual preference as indexed by eye tracking are hallmarks; however, whether they can be used to define an early biomarker of ASD as a whole or leveraged to define a subtype is unclear. To begin to examine this issue, large cohorts are required.

METHODS: A sample of 334 toddlers from six distinct groups (115 toddlers with ASD, 20 toddlers with ASD features, 57 toddlers with developmental delay, 53 toddlers with other conditions [e.g., premature birth, prenatal drug exposure], 64 toddlers with typical development, and 25 unaffected toddlers with siblings with ASD) was studied. Toddlers watched a movie containing geometric and social images. Fixation duration and number of saccades within each area of interest and validation statistics for this independent sample were computed. Next, to maximize power, data from our previous study ($n = 110$) were added for a total of 444 subjects. A subset of toddlers repeated the eye-tracking procedure.

RESULTS: As in the original study, a subset of toddlers with ASD fixated on geometric images >69% of the time. Using this cutoff, sensitivity for ASD was 21%, specificity was 98%, and positive predictive value was 86%. Toddlers with ASD who strongly preferred geometric images had 1) worse cognitive, language, and social skills relative to toddlers with ASD who strongly preferred social images and 2) fewer saccades when viewing geometric images. Unaffected siblings of ASD probands did not show evidence of heightened preference for geometric images. Test-retest reliability was good. Examination of age effects suggested that this test may not be appropriate with children >4 years old.

CONCLUSIONS: Enhanced visual preference for geometric repetition may be an early developmental biomarker of an ASD subtype with more severe symptoms.

Keywords: Autism spectrum disorder, Early detection, Eye gaze, Eye tracking, Geometric preference, Visual attention

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Robust biomarkers of autism spectrum disorder (ASD) in infants and toddlers have yet to be discovered, perhaps due to the considerable clinical, and likely etiological, heterogeneity associated with ASD (1). For example, some children with ASD have high verbal competency, whereas others may not speak at all; some excel in response to treatment, whereas others do not (2,3). Complicating ASD further is the fact that symptom onset is quite variable—some toddlers may show signs at or even before the first birthday, whereas others may not show signs until the second birthday or later (4). Biomarkers are generally conceptualized as measurable indicators of normal or pathologic biological processes (5), and we use the term in this article to refer to any objective indicator that accurately and reliably identifies ASD or a subtype of ASD.

At a diagnostic level, the urgency to discover early developmental biomarkers is mediated by the belief that a valid early biomarker might hasten the pace of diagnosis and the interval between first diagnosis and eventual treatment (6). In theory, early treatment can affect functional connections in the developing brain and lead to improved outcomes for children (7,8). For example, a study showed that toddlers who began behavioral treatment before age 3 years, including several by age 18 months, experienced a gain of 15 points on a standardized IQ test after treatment (9).

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At a prognostic level, biomarkers might be able to act as a specifier to diagnosis, generating a more in-depth characterization of a child's overall clinical profile that may relate to his or her long-term outcome. At the treatment level, biomarkers might be able to identify subgroups of toddlers with ASD who could be matched to specific interventions tailored for that specific subtype. Although identifying toddlers with ASD as early as possible is critical, the heterogeneity of ASD suggests that studies might productively target discovery of clearly definable subtypes of toddlers with ASD.

Biomarker tests of ASD need to be developmentally appropriate, while having the potential to detect features before manifestation of symptoms. Unusual patterns of visual attention are emerging as preclinical markers in ASD. In general, toddlers with ASD fail to attend to social attention cues (10) or may display "sticky attention" (11). Eye-tracking technology may be ideally suited to tap into such abnormalities because it is easily implemented, it is objective, and it can be used from infancy to adulthood. Eye-tracking studies with toddlers <3 years old with ASD highlighted a range of social visual attention deficits, such as a reduced preference for biological motion (12), reduced fixation to eye (13,14) and head regions (15), difficulties in joint attention (16), and scene monitoring during explicit dyadic cues (17). Although some of these findings have been questioned (18), collectively these studies point to very early developmental origins of social dysfunction in ASD.

Despite their promise, effects reported in eye-tracking studies are often subtle and mainly applicable at the group, not individual subject, level. Further, validation statistics needed to translate eye-tracking metrics into usable biomarkers, such as specificity, positive predictive value (PPV), and negative predictive value (NPV), are generally not reported. One eye-tracking study attempted individual-level classification based on the hypothesis that reduced fixation toward the eye region at 6 months old would predict diagnostic status at 24–36 months old but found that this measure did not accurately classify individual toddlers as having ASD (19). However, a more recent baby-sibling design study that intensively tracked 11 infants with ASD from 2 to 24 months suggested that at 6 months of age fixations toward the eye region are just beginning to decline, with maximal reductions in eye region fixation not occurring until 2 years (14). It may be the case that only after the 6-month age point can diagnostic classification efforts be maximally successful.

To discover the utility of potential biomarkers using eye tracking, large samples need to be examined so that validation statistics such as sensitivity and specificity can be computed and potential subgroups can be identified across a wide age range. We previously developed a novel implementation of a traditional preferential looking paradigm using eye-tracking technology (20). We quantified visual attention preferences of toddlers with ASD and typically developing toddlers toward dynamic (i.e., moving and changing) geometric images (DGI) versus dynamic social images (DSI) and identified a unique subgroup of toddlers with ASD who strongly preferred to look at geometric rather than social images. Using this GeoPref Test, behavior of individual subjects with ASD could predict diagnostic classification (20). Specifically, the study found that toddlers who visually fixated on DGI >69% of the time had a 100% probability of having ASD. However, sensitivity was modest capturing 20%–40% of toddlers with ASD depending on the threshold used. The GeoPref Test showed initial signs of promise at detecting specific ASD subgroups.

Our original study did not completely address whether or not the geometric preference effect was specific to ASD or might also be found in toddlers with other language and cognitive delays. The study also was too small to address fully the question of whether this ASD subgroup might have distinctive clinical characteristics or whether the geometric preference effect varied with age or with repeat testing at shorter or longer intervals. Lastly, it did not include information relevant to the search for ASD endophenotypes—that is, whether this biomarker is shared by nonaffected siblings and so could be used in future studies of underlying genetic factors.

In the present study, we addressed these issues by first testing and replicating the effect in 334 toddlers completely independent from the original study and including multiple contrast groups including unaffected siblings of ASD probands in our sample. We next combined data from the original study of 110 toddlers with this sample to arrive at an overall data set of 444 toddlers, producing the largest eye-tracking study of ASD to date.

METHODS AND MATERIALS

Participants

Identical to the approach used in our earlier study (20), toddlers were recruited through two mechanisms: community referrals (e.g., website) or a general population-based screening method called the 1-Year Well-Baby Check-Up Approach (21) that allowed for the prospective study of ASD beginning at 12 months based on a toddler's failure of the CSBS-DP Infant-Toddler Checklist (22,23).

Participation of 424 toddlers aged 10–49 months was attempted. There were 90 toddlers (21.2%) excluded for multiple reasons including a failure to attend to at least 50% of the video, a criterion that was adopted in our previous study (20) (Supplement and Supplemental Figures S1 and S2). The final sample consisted of 334 toddlers and was nonoverlapping and independent from the sample included in our prior study (20).

All assessments were administered by licensed, Ph.D.-level psychologists blind to eye-tracking results. All toddlers, including normal control subjects, participated in a series of tests including the Autism Diagnostic Observation Schedule (ADOS; Module T, 1, or 2) (24), the Mullen Scales of Early Learning (25), the Vineland Adaptive Behavior Scales (26), and other tests as part of a larger study (see www.autism-center.ucsd.edu) at every test visit. Table 1 presents characteristics of the independent sample. All testing occurred at the University of California San Diego Autism Center.

Based on diagnoses given at the final diagnosis age, the independent sample of 334 toddlers consisted of six discrete diagnostic groups (115 toddlers in ASD group, 20 toddlers in ASD-Feat group, 57 toddlers in DD group, 53 toddlers in Other group, 64 toddlers in TD group, and 25 toddlers in Typ Sib group). The ASD group included toddlers with a final diagnosis

Table 1. Characteristics of Subjects for the Independent Sample (n = 334)[a]

Characteristic	Mean (SD) [Range]						*p* Value				
	ASD (n = 115)	ASD-Feat (n = 20)	TD (n = 64)	DD (n = 57)	Typ Sib ASD (n = 25)	Other (n = 53)	ASD vs. ASD-Feat	ASD vs. TD	ASD vs. DD	ASD vs. Typ Sib	ASD vs. Other
Sex, M/F	88/27	15/5	35/29	45/12	12/13	26/27	.884	.004	.723	.014	.001
Age, Months	28.0 (8.4) [12–49]	22.2 (9.3) [11–42]	23.6 (9.9) [12–44]	22.0 (8.3) [10–46]	19.1 (6.0) [12–31]	22.0 (8.7) [12–43]	.006	.004	<.001	<.001	<.001
Mullen Scales (*t* Scores)											
Visual Reception	42.3 (12.9)	49.7 (11.7)	59.0 (9.1)	50.6 (9.5)	57.3 (9.4)	55.0 (11.3)	.020	<.001	<.001	<.001	<.001
Fine Motor	39.4 (12.8)	47.4 (13.1)	57.7 (8.3)	51.5 (8.9)	58.6 (7.4)	55.5 (10.7)	.012	<.001	<.001	<.001	<.001
Receptive Language	32.3 (13.2)	46.2 (11.8)	54.6 (9.2)	43.8 (11.4)	51.6 (8.6)	51.7 (11.9)	<.001	<.001	<.001	<.001	<.001
Expressive Language	32.0 (13.0)	42.0 (12.7)	52.8 (8.4)	39.4 (9.9)	53.2 (10.1)	46.7 (10.8)	.002	<.001	<.001	<.001	<.001
Early Learning Composite[b]	77.0 (19.8)	93.3 (18.2)	112.5 (11.3)	93.7 (13.9)	111.6 (12.5)	104.5 (15.9)	.001	<.001	<.001	<.001	<.001
Vineland (Standard Scores)											
Communication	81.2 (13.4)	90.1 (12.4)	102.4 (9.8)	90.3 (10.7)	99.0 (9.8)	94.9 (11.5)	.006	<.001	<.001	<.001	<.001
Daily Living	86.5 (11.6)	87.5 (16.5)	100.7 (9.2)	92.5 (12.1)	99.1 (11.7)	96.6 (11.8)	.731	<.001	.002	<.001	<.001
Socialization	84.3 (12.1)	91.8 (9.9)	103.3 (8.5)	96.8 (7.5)	102.2 (8.1)	98.6 (9.9)	.009	<.001	<.001	<.001	<.001
Motor Skills	91.0 (11.1)	93.6 (12.2)	101.1 (7.5)	96.88 (9.3)	101.8 (9.4)	95.9 (11.8)	.350	<.001	.001	<.001	.010
Adaptive Behavior Composite	82.4 (13.4)	87.0 (15.1)	102.0 (8.5)	92.9 (8.1)	100.4 (8.7)	95.5 (10.9)	.170	<.001	<.001	<.001	<.001
ADOS[c] (Module T, 1, or 2)											
ADOS SA/CoSo score	13.6 (4.7)	7.7 (4.6)	1.6 (1.6)	3.6 (2.9)	2.2 (2.5)	4.0 (3.5)	<.001	<.001	<.001	<.001	<.001
ADOS RRB score	3.6 (1.9)	2.0 (1.5)	.1 (.4)	.6 (.8)	.4 (.8)	.7 (1.0)	<.001	<.001	<.001	<.001	<.001
ADOS total score	17.2 (5.9)	9.65 (4.81)	1.8 (1.7)	4.4 (3.1)	2.6 (2.7)	4.7 (3.9)	<.001	<.001	<.001	<.001	<.001

ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CoSo, Communication Social Score; M/F, male/female; RRB, Restricted and Repetitive Behavior; SA, Social Affect.

[a]See text for descriptions of diagnostic groups ASD, ASD-Feat, TD, DD, Typ Sib, and Other.

[b]6% of the sample received Wechsler Preschool and Primary Scale of Intelligence instead of Mullen scale.

[c]Administered ADOS module depended on the age and language ability of the toddler at the time of testing.

of ASD, the ASD-Feat group included toddlers with ASD symptoms but who did not meet full diagnostic criteria, the DD group included toddlers with either a language or global developmental delay, the Other group included toddlers with a wide array of conditions such as premature birth or prenatal drug exposure, the TD group included toddlers with typical development, and the Typ Sib group included unaffected toddlers with siblings with ASD (Supplement). Following replication tests of the original finding, the current sample (n = 334) was combined with the original sample (n = 110) (20) to capitalize on increased power to detect eye-tracking behavior effects (combined N = 444 subjects; 152 ASD, 20 ASD-Feat, 79 DD, 53 Other, 115 TD, and 25 Typ Sib).

Apparatus, Movie, and Eye-Tracking Procedure

A Tobii T120 Eye Tracker with a 17-inch thin-film transistor monitor (Tobii, Stockholm, Sweden; www.tobii.com) was used to measure toddlers' fixations and number of saccades in response to a visual stimulus. Toddlers were presented with a movie consisting of two rectangular areas of interest that contained DGI and DSI placed side by side in which scenes changed in a simultaneous, time-linked fashion identical to our previous experiment (20). Audio information was not presented. The final movie contained 28 scenes with single-scene duration varying from 2 to 4 seconds for a total presentation time of 60 seconds. The side (left/right) of presentation of DGI and DSI scenes was randomly assigned across subject and diagnosis (Supplement).

Statistical Analyses

Visual Attention as Indexed by Fixation Time and Relation to Clinical Characteristics and Age. Using Tobii software, fixation data were calculated using a 35-pixel radius filter. Percent time spent fixating within each area of interest (i.e., DGI or DSI) was tabulated for each subject. To compare percent fixation time within DGI between groups, a one-way analysis of covariance (ANCOVA) was performed with six levels (diagnostic groups) using the age of the child at testing as a covariate. Significant effects were followed by planned contrasts with Bonferroni correction for multiple comparisons, and 95% confidence interval (CI) of the mean difference between

groups and effect sizes was reported. Examinations of the relationship between percent fixation on DGI and clinical measures were conducted using linear regression controlling for the effects of age. To examine if clinical symptoms were more or less severe in toddlers with ASD who strongly preferred DGI (i.e., ≥69% toward DGI) relative to toddlers with ASD who preferred DSI (i.e., ≥69% toward DSI), clinical characteristics of toddlers within each subgroup were directly compared using ANCOVA controlling for age.

Sensitivity, Specificity, PPV, NPV, and Receiver Operating Characteristic Curve Analyses. To determine the specific percentage of fixation time within DGI that would best discriminate toddlers with ASD from other toddlers, a receiver operating characteristic curve was generated that graphically displayed a plot of the true positives versus false positives. With respect to PPV and NPV, two methods were used: 1) PPV and NPV were calculated within the study sample, as would be applicable in a second-tier screening approach; 2) PPV and NPV were calculated taking into account the ASD prevalence rate of 1.47% in the general population (27), as would be applicable in a first-tier screening approach.

Number of Saccades. The number of saccades per second was determined for each subject by dividing the overall total number of saccades by the total looking time. Differences in saccade data between diagnostic groups were examined using a one-way ANCOVA covarying for age and planned contrasts.

Test-Retest Reliability. To determine the stability of the GeoPref Test, 208 toddlers (61 ASD, 9 ASD-Feat, 37 DD, 28 Other, 63 TD, and 10 Typ Sib) 12–48 months old participated in a retest session within 1 hour to 24 months following their first GeoPref Test (Supplement).

Fixation Patterns Between Sibling Pairs (Exploratory). Data were available from 36 sibling pairs (11 concordant for ASD, 12 discordant for ASD, and 13 typical sibling pairs). An intraclass correlation coefficient was used as an exploratory analysis to determine the degree to which sibling pairs resembled each other in terms of their preference for DGI (Supplement).

RESULTS

Differences in Visual Preference Patterns Toward DGI Between Diagnostic Groups

Independent Sample (n = 334). Within the independent sample, differences in percent fixation toward DGI were found using an ANCOVA controlling for the age of participants ($F_{6,327}$ = 16.39, $p < .001$, partial η^2 = .23), replicating our previous findings (20). Follow-up contrasts comparing the ASD group with each of the other groups, with Bonferroni correction and examination of the CI of the difference in percent fixation between groups, revealed that toddlers with ASD had significantly greater percent fixation on DGI than all other diagnostic groups (ASD vs. DD, $p < .001$, CI = 9.32%–22.33% fixation, Cohen's d = .77; ASD vs. Other, p = .004, CI = 3.09%–16.39% fixation, Cohen's d = .50; ASD vs. TD, $p < .001$, CI = 13.46%–25.84% fixation, Cohen's d = .97; ASD vs. Typ Sib, $p < .001$, CI = 14.19%–31.99% fixation, Cohen's d = .92) except the ASD-Feat group (ASD vs. ASD-Feat, p = .33, CI = −4.83% to 14.32% fixation, Cohen's d = .23). See Figure 1 and videos in the Supplement for examples and the Supplement for total looking time within each group.

Combined Sample (N = 444). Within the combined sample, the exact same pattern was observed in differences to DGI fixation percentages controlling for the age of participants ($F_{6,437}$ = 20.23, $p < .001$, partial η^2 = .22). See the Supplement for follow-up contrasts.

Sensitivity, Specificity, PPV, NPV, and Receiver Operating Characteristic Curves

Using the 69% fixation threshold (i.e., a toddler fixated on geometric shapes ≥69% of the time), validation statistics found within the independent sample were almost identical to the previous study with sensitivity 21%, specificity 98%, PPV 86%, and NPV 70%. This almost identical replication suggests that the GeoPref Test accurately identifies a select and highly stable subtype within the autism spectrum.

The tradeoffs between sensitivity and specificity of the GeoPref Test are illustrated further in Figure 2 and associated tables that contain validation statistics for the independent sample (n = 334) and the combined sample (N = 444) (Table 2). Validation statistics of the GeoPref Test can change based on the specific cutoff threshold used and whether or not toddlers with ASD features are considered true positives. For example, the sensitivity of the test can be improved by lowering the fixation threshold to 50%. Results of the GeoPref Test changed only slightly if toddlers in the ASD-Feat group were included as true positives. For more information regarding validation statistics within narrow age bins (e.g., 24–30 months), see Supplemental Table S3 and Figure S5.

Relationship Between DGI Fixation, Age, and ASD Symptoms

Combined Sample (N = 444). Given that the pattern of effects and effect sizes from the independent and combined samples were very similar, we examined further relationships with the combined sample to increase power (although the relationships do not change if examined only in the independent sample). Overall, among all participants, a significant relationship was found between DGI percent fixation and age (r = .26, $p < .001$), justifying age as a covariate in our main analyses. Because of different age distributions between each diagnostic group, age effects were examined for each group separately. As indicated in Figure 3, toddlers from the ASD, Other, and TD groups tended to fixate more on DGI with increasing age, suggesting that the GeoPref Test may be less effective with older children (i.e., >4 years old).

Linear regressions within each group with symptom rating scales as the dependent variables and DGI percent fixation and age as explanatory variables demonstrated that the degree to which a toddler fixated on DGI, independent of age, was

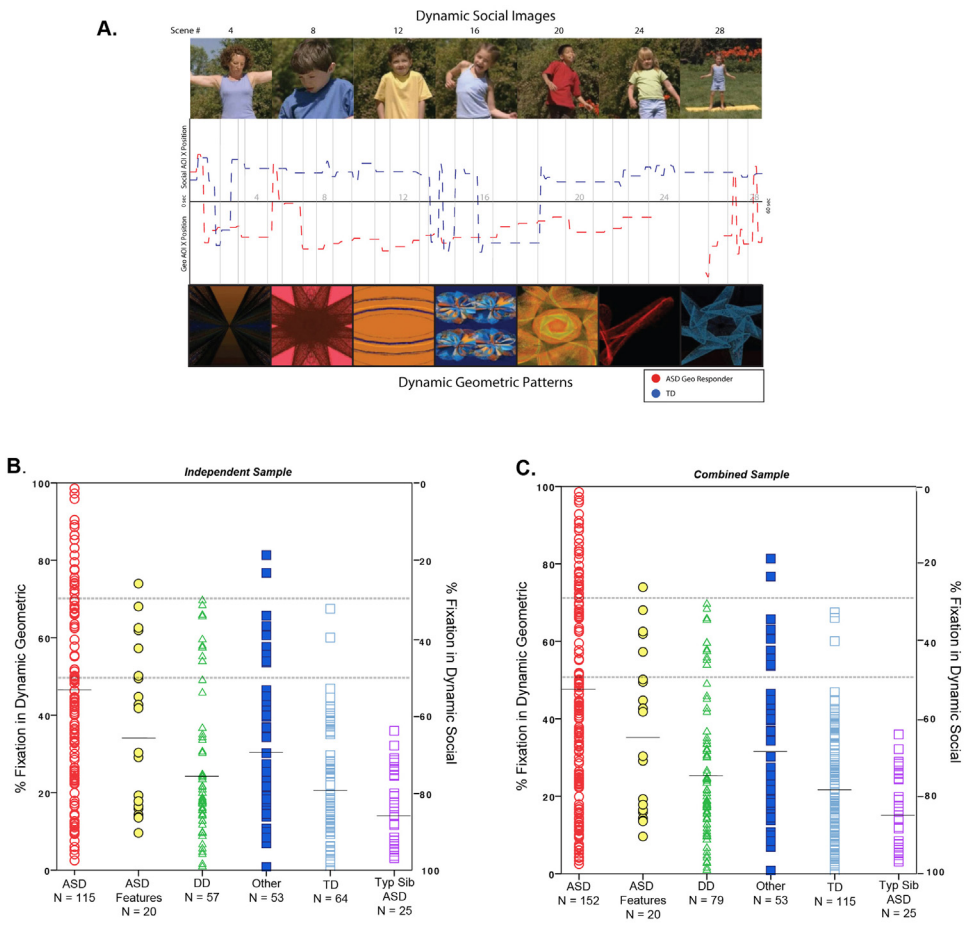

Figure 1. **(A)** Sample social and geometric scenes from the 1-minute (28-scene) GeoPref Test. Visual scanning data (x-axis fixation points) from a typically developing toddler (blue line) and a geometric responder toddler with autism spectrum disorder (red line) are plotted across time. Breaks in the line represent a lack of fixation toward the movie. **(B)** Scatterplot of the independent group (n = 334) illustrating the percentage of fixation time to dynamic geometric images and dynamic social images for each toddler across each diagnostic group (see text for diagnostic groups). Total percent time viewing dynamic geometric images and dynamic social images sums to 100% for each toddler. A toddler who spends 80% viewing time on geometric images (as noted on the y-axis on the left) thus spends 20% viewing time on social images (as noted on the y-axis on the right). A toddler who spends >50% viewing geometric images is considered a geometric responder, and a toddler who spends >50% viewing social images is considered a social responder. **(C)** Scatterplot of the combined group (N = 444). AOI, area of interest; ASD, autism spectrum disorder; TD, typical development.

significantly related to his or her symptom severity across a range of areas. such as receptive and expressive language ability, cognition, autism symptom profile, and adaptive functioning, controlling for age. For example, as illustrated in Table 3, a 1% increase in fixation of a child with ASD toward geometric images was associated with a .29 reduction in his or her expressive language ability. In contrast, no significant relationships were found between DGI percent fixation and test scores within any other group when accounting for the age of the child and when not accounting for the age of the child.

ASD Geometric Responders Versus ASD Social Responders. The toddlers with ASD in our sample reflected a wide range of visual preference patterns; some toddlers strongly preferred DGI (defined as DGI fixation levels ≥69%), whereas others strongly preferred DSI (defined as DSI fixation levels ≥69%). Using an ANCOVA with age as a covariate, direct comparisons between these two subgroups revealed differences in Visual Reception ($F_{2,84}$ = 12.26, p < .001, partial η^2 = .23), Receptive Language ($F_{2,84}$ = 9.70, p < .001, partial η^2 = .19), Expressive Language ($F_{2,84}$ = 6.60, p = .002, partial η^2 = .14), and the Early Learning Composite Score ($F_{2,84}$ = 9.17, p < .001, partial η^2 = .18) based on scores from the Mullen Scales of Early Learning. Similarly, significant differences were found in the Daily Living subscale ($F_{2,86}$ = 10.86, p < .001, partial η^2 = .20), Socialization subscale ($F_{2,84}$ = 18.23, p < .001, partial η^2 = .30), Communication subscale ($F_{2,84}$ = 5.61, p = .005, partial η^2 = .12), and Adaptive Behavior Composite ($F_{2,84}$ = 13.97, p < .001, partial η^2 = .25) between geometric responders with ASD and social responders with ASD. Finally, significant differences also were found between these two ASD subtypes on the ADOS Social Affect/Communication scale ($F_{2,86}$ = 8.20, p = .001, partial η^2 = .16) and total ADOS score ($F_{2,86}$ = 5.76, p = .004, partial η^2 = .12). Collectively, results showed that toddlers with ASD with the geometric responder profile had worse scores on every test compared with toddlers with ASD with the social responder profile (Figure 4).

Unique Saccade Pattern in Toddlers With ASD Who Preferred Geometric Images

Our previous study split toddlers with ASD into two groups based on the middle point of the GeoPref Test (i.e., 50% fixation) and demonstrated that toddlers with ASD who preferred DGI (defined as DGI fixation levels ≥50%) exhibited fewer saccades when looking at geometric images than all other toddlers (20). To replicate these findings, we performed similar analyses in the present. Similar to percent time fixating on DGI, an ANCOVA controlling for age demonstrated a main

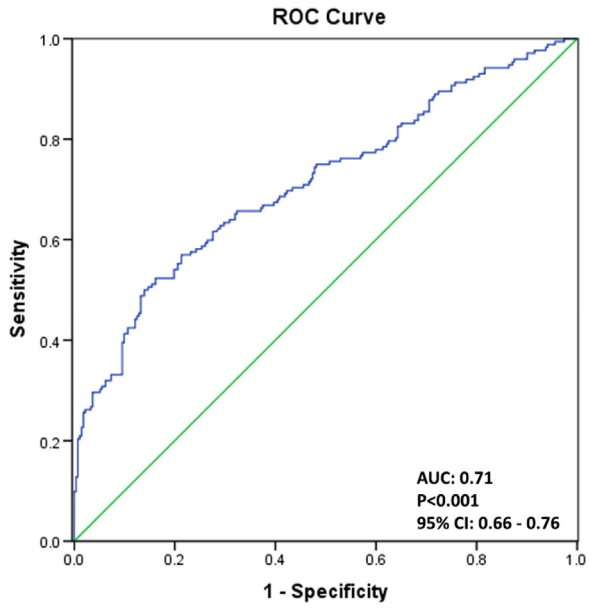

Figure 2. Receiver operating characteristic curve plot graphically illustrating the true-positive rate (sensitivity) vs. the false-positive rate (1 − specificity) of the combined (N = 444) sample. AUC, area under curve; CI, confidence interval; ROC, receiver operating characteristic.

effect of diagnosis in the number of saccades per seconds while viewing DGI ($F_{7,433}$ = 4.12, $p < .001$, partial η^2 = .06). Contrasts revealed that toddlers with ASD who were geometric responders exhibited significantly fewer saccades (i.e., 1.33 saccades/sec) and longer bouts of sustained attention when they were viewing DGI relative to toddlers with ASD who were social responders (1.9 saccades/sec, $p < .001$, Cohen's d = .76) and toddlers in the TD (1.84 saccades/sec, $p < .001$, Cohen's d = .71), DD (1.91 saccades/sec, $p < .001$, Cohen's d = .70), Typ Sibs (1.89 saccades/sec, p = .002, Cohen's d = .82), and Other (1.91 saccades/sec, $p < .001$, Cohen's d = .91) groups. However, there were no significant differences between toddlers with ASD and toddlers in the ASD-Feat group (1.68 saccades/sec; p = .068, Cohen's d = .47). In contrast, when the geometric responders with ASD viewed their nonpreferred stimuli—the social stimuli—they exhibited a significantly greater number of saccades relative to other contrast groups (all $p < .001$) (Figure 5).

Test-Retest Reliability

A visual preference for DGI, or lack thereof, appears to be a largely stable phenomenon. However, test-retest reliability was stronger with immediate retest (i.e., within 1 month of original test session; intraclass correlation coefficient = .84, $p < .001$) compared with later (i.e., >1 year past original test session; intraclass correlation coefficient = .52, $p < .01$) (Supplement and Supplemental Tables S1 and S2 and Figure S4).

Fixation Patterns Between Sibling Pairs (Exploratory)

Patterns of visual fixation were significantly correlated in siblings concordant for ASD but not in other sibling groups (Supplement and Supplemental Table S4).

Table 2. Validation Statistics for Independent and Combined Samples

	Using 50% Geometric Fixation Cutoff (ASD Only as TP)	Using 50% Geometric Fixation Cutoff (ASD + ASD Features as TP)	Using 69% Geometric Fixation Cutoff (ASD Only as TP)	Using 69% Geometric Fixation Cutoff (ASD + ASD Features as TP)
Independent Validation Sample (n = 334)				
TP	43	49	24	25
TN	190	176	215	196
FP	29	23	4	3
FN	72	86	91	110
Sensitivity	37%	36%	21%	19%
Specificity	87%	88%	98%	99%
PPV	60%	68%	86%	89%
NPV	73%	67%	70%	64%
Combined Sample (N = 444)				
TP	58	64	35	36
TN	260	246	288	269
FP	32	26	4	3
FN	94	108	117	136
Sensitivity	38%	37%	23%	21%
Specificity	89%	90%	99%	99%
PPV	64%	71%	90%	92%
NPV	73%	70%	71%	66%

ASD, autism spectrum disorder; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

DISCUSSION

In infants, there is a near imperative to attend preferentially to the human face and social stimuli over nonsocial stimuli, even within minutes after birth (28). However, as investigated in the present study, a subgroup of toddlers with ASD (~20%) do not show this preference. Instead, toddlers with this unique subtype of ASD prefer to examine DGI visually. A strong preference for moving geometric images over social images was highly specific to this ASD subtype compared with toddlers with typical development, language delay, and global developmental delay as well as unaffected siblings of toddlers with ASD.

With specificity levels of 98%, the GeoPref test was able to signify ASD status in a subset of individual toddlers with very high accuracy and may be more powerful than other biomarker attempts at the behavioral (29–31), genetic (32–34), and neuroimaging (35–37) levels. Eye-tracking technology is attractive as a potential tool in early identification and clinical evaluation efforts because patterns of eye gaze are objective, quantifiable behaviors based on neural systems known to be abnormal in ASD such as the visual attention system (38–40).

It has been clear since Kanner's original definition of autism in the 1940s (41) that children with autism do not visually attend to cues in their environment, including social and nonsocial cues, to the same degree or in the same way as

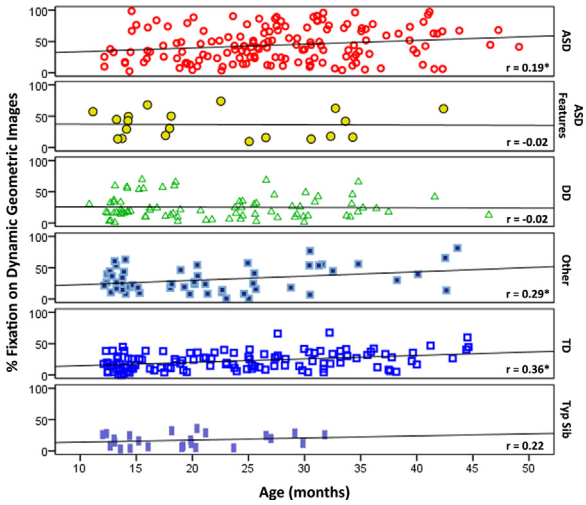

Figure 3. Scatterplots with a best-fit line illustrating the relationship between percent fixation toward dynamic geometric images and age across each diagnostic group (see text for diagnostic groups). *$p < .05$.

typically developing children (41–51). It is less clear whether unusual patterns of preferential visual attention in ASD are sequelae resulting from having the disorder across time or are primary and early emerging. New studies have shown that baby siblings of ASD probands who later test positive for ASD display "sticky attention" or periods of abnormally prolonged visual fixation at 12 months of age (11) and that fixations within the eye region begin to decline abnormally after 6 months (14). In the present study, the toddlers with ASD and with a geometric preference, many 12–24 months old, not only showed abnormalities in what they preferred to look at but also produced significantly fewer saccades while viewing geometric shapes than all other toddlers. Collectively, our study combined with studies of other authors (11,14) suggests that abnormalities in visual attention and preference are among the earliest emerging warning signs of ASD, and although this may be reflective of very early neural circuit organization abnormalities, experience-dependent mechanisms likely also play a role in the development of abnormal visual attention in ASD across the first years of life (52,53).

Results of the present study go deeper than the pressing need to discover biomarkers of ASD that will hasten early detection and treatment. Beyond this goal is the knowledge that some toddlers with ASD, no matter how many tests are administered or how early treatment is started, will do well in life, whereas others may not even learn to speak. Understanding factors relating to outcome and prognosis in children with ASD are among the most important goals in the field of autism today (54–58). Although research has shown that 25% of children with ASD may no longer meet the diagnostic criteria of ASD at some point (58), very little is known about factors that might predict later outcome at the time of diagnosis or before that time. In the present study, toddlers with the most intense fixation toward geometric images were also the subjects with the most severe ASD symptoms, worst language, and lowest overall IQ scores. Our initial data thus suggest that what a toddler prefers to examine visually may be a valuable prognostic marker. Toddlers with ASD who are geometric responders not only need to be identified very early but also may require unique, yet to be determined, treatment approaches. Conversely, toddlers with ASD with intense fixation toward social images had better language, higher IQ score, and fewer symptoms compared with the toddlers with ASD who were geometric responders. It is conceivable, although yet to be empirically verified, that toddlers with ASD who are very strong social responders at early ages will experience more positive long-term outcomes.

Although heightened visual fixation toward geometric images may represent a unique subgroup of toddlers with ASD, this trait is likely unsuitable for the label "endophenotype," which, according to current definition, requires the trait to be heritable and present in unaffected family members (59). Fixation toward geometric images was actually lower in unaffected siblings of ASD probands relative to normal control controls, suggesting a protective mechanism, possibly genetic, for unaffected siblings. However, there is reason to suggest that an intense fixation toward geometric images might be at least partially genetically driven in that sibling pairs concordant for ASD showed the highest correlation in visual attention toward geometric images, whereas no significant correlation was found between typically developing sibling pairs or between sibling pairs where only one sib had ASD.

Although the GeoPref Test was highly accurate in identifying a subset of true-positive ASD cases and had good test-retest reliability performance, the overall sensitivity of the test to detect all ASD cases fell within the range of 20%–40%, depending on the cutoff used. However, it would be a misunderstanding of ASD to assume any single test would detect all ASD cases. Single biomarkers, such as heightened visual preference for repetitive geometric images, are limited in their ability to capture and parse all of the heterogeneity and complexity of a multifactorial disorder such as ASD. Although percent fixation levels toward geometric images were highest in the ASD group, it was not significantly different between toddlers with a final ASD diagnosis and toddlers who showed ASD features only, further underscoring the dimensionality of the disorder. Although it is generally believed that autism has a strong genetic component (60,61), studies also suggest that nongenetic, currently unknown environmental factors may account for up to 50% of the total variance (61). It may be the case that combining multiple measures with low sensitivity but extremely high accuracy and specificity, such as the GeoPref Test, will be an effective method for detecting a larger portion of the highly heterogeneous ASD population in the future.

Findings showed that all toddlers—ASD, TD, and contrast cases alike—visually fixated on geometric images more strongly with increasing age. This change in visual fixation is likely due to the fact that during very early development, typically developing infants and toddlers are strongly drawn to the human face (28), whereas other factors, such as an increased response to novelty, emerge during early childhood that may have an impact on the ability of the GeoPref Test to discriminate ASD from other disorders at older ages.

In terms of the predictive ability of the GeoPref Test to identify children with ASD correctly, we found high values in classifying children with ASD within our sample (i.e., 90% PPV

Table 3. Coefficient and *r* Values Based on Linear Regression Demonstrate Relationship Between Percent Fixation Toward Dynamic Geometric Images and Social, Cognitive, and Language Skills Within ASD Group

Test Name	Coefficient	SE	Partial *r*	*p* Value
Mullen Scales of Early Learning				
Visual Reception	−.229	.036	−.237	.004
Receptive Language	−.299	.039	−.295	<.001
Expressive Language	−.291	.038	−.290	<.001
Early Learning Composite	−.219	.056	−.306	<.001
Vineland Adaptive Behavior Scales				
Communication	−.140	.040	−.273	.001
Daily Living	−.083	.035	−.193	.018
Socialization	−.112	.033	−.266	.001
Adaptive Behavior Composite	−.114	.038	−.238	.003
ADOS				
ADOS SA/CoSo score	.353	.014	.349	<.001
ADOS RRB score	.46	.006	.046	.578
ADOS Total score	.306	.017	.302	<.001

All regression models include percent geometric fixation and age as variables. Partial *r* expresses the unique variance accounted for in clinical measures by percent fixation to geometric images among children with ASD.

ADOS, Autism Diagnostic Observation Schedule, ASD, autism spectrum disorder; CoSo, Communication Social Score; RRB, Restricted and Repetitive Behavior; SA, Social Affect.

when using 69% fixation cutoff). However, our sample, as with all research samples, does not reflect the base rate of ASD in the general population. Instead, in the present study, most participants were referred for eye tracking after they failed a broadband developmental screen (i.e., the CSBS) at their pediatrician's office or after a delay was suspected by a parent or health care provider (21). As such, eye tracking as applied to our sample is highly reflective of a second-tier screening approach. When considering eye tracking as a possible first-tier screening approach, PPV and NPV should be calculated using ASD population base rates. In this case, PPV decreases to 17%, as only ~1.4 out of 100 children will develop ASD (27). Changes to the PPV as reflected in a second-tier or first-tier screening approach highlight the importance of the population and setting in which a classification test will be used (62). Moreover, our study was limited in the sense that the number of normal control participants was arbitrary in count, which could also affect estimates of PPV and NPV.

At this stage of the science, the presence of an ASD biomarker in a toddler, such as heightened visual preference for dynamic geometric images, should not replace clinical diagnosis. Rather, the presence of this trait has considerable scientific and clinical value as an early biomarker of ASD that could hasten the pace of early identification and treatment and provide valuable prognostic information. Toddlers who show a preference for geometric images have worse symptoms than other toddlers with ASD and may have distinctive underlying neurobiological attributes. It is conceivable, although not yet empirically tested, that the subtype of toddlers with ASD who have the geometric responder profile may require specific treatments tailored to their unique biology.

In conclusion, although it is unclear what the future will hold, it is possible that diagnosis may move away from a purely clinical judgment DSM approach to a more objective research domain approach that instead focuses on the

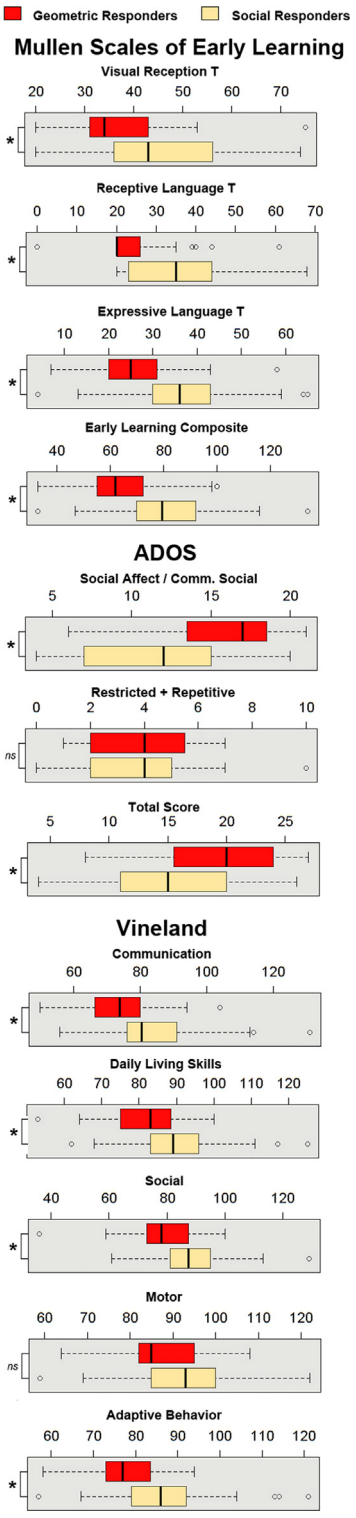

Figure 4. Box-and-whisker plot representing distribution of Mullen, Autism Diagnostic Observation Schedule, and Vineland scores for toddlers with ≥69% fixation toward dynamic geometric images (i.e., geometric responders, $n = 35$, red box) and ≥69% fixation toward dynamic social images (i.e., social responders, $n = 54$, tan box). The black line in the center of each box represents the median; the top and bottom of each box represent the first and third quartiles, respectively; and the whiskers represent the minimum and maximum of the data. Outliers are represented as circles. Scores were significantly different between toddlers with autism spectrum disorder who were geometric responders and toddlers with autism spectrum disorder who were social responders for all test subscales, with the exception of motor on the Vineland and the restricted and repetitive on the Autism Diagnostic Observation Schedule. ADOS, Autism Diagnostic Observation Schedule.

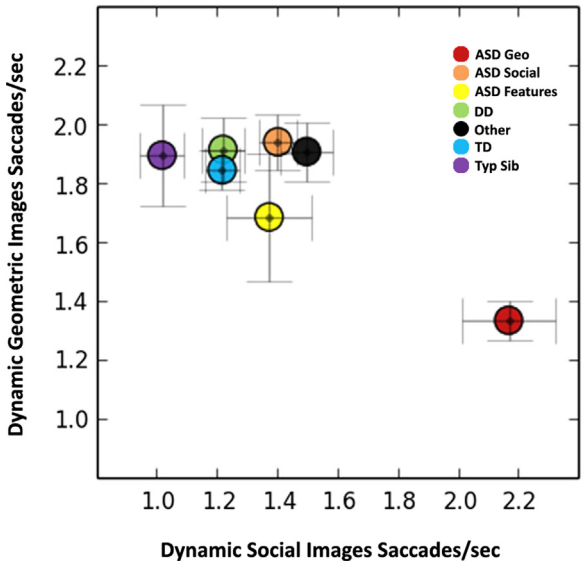

Figure 5. Plot illustrating the average number of saccades per second when toddlers were viewing dynamic geometric images or dynamic social images for each diagnostic group (see text for diagnostic groups). Toddlers with autism spectrum disorder who displayed a geometric responder profile were plotted separately from toddlers with autism spectrum disorder who displayed a social responder profile. The toddlers with autism spectrum disorder who were geometric responders (red circle, bottom right of graph) displayed a unique saccade pattern that included fewer saccades when fixating on geometric shapes and more saccades when viewing social images. Error bars represent SEM. Dynamic geometric image saccades/sec means ± SE for diagnostic groups were as follows: ASD Geo, 1.33 ± .07; ASD Social, 1.94 ± .09; ASD Features, 1.68 ± .22; DD, 1.91 ± .11; Other, 1.91 ± .10; TD, 1.86 ± .07; Typ Sib, 1.89 ± .17. Dynamic social image saccades/sec means ± SE for diagnostic groups were as follows: ASD Geo, 2.17 ± .16; ASD Social, 1.40 ± .06; ASD Features, 1.37 ± .14; DD, 1.22 ± .07; Other, 1.50 ± .09; TD, 1.22 ± .06; Typ Sib, 1.02 ± .07. ASD Geo, toddlers with autism spectrum disorder who were geometric responders; ASD Social, toddlers with autism spectrum disorder who were social responders.

measurement of reproducible biological traits (63,64). Large adequately powered research studies such as the present study are essential to enable definition of clinically relevant biomarkers and comparison of biomarker levels across different diagnostic groups.

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ARTICLE INFORMATION

From the Departments of Neurosciences (KP, SM, RH, CCB, AM) and Psychiatry (BM), University of California, San Diego, La Jolla, California.

Address correspondence to Karen Pierce, Ph.D., Autism Center of Excellence, 8110 La Jolla Shores Drive, La Jolla, CA, 92037; E-mail: kpierce@ucsd.edu.

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