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Title: Uncovering Neurodevelopmental Paths to Autism Spectrum Disorder through an Integrated Analysis of Developmental Measures and Neural Sensitivity to Faces.

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Abstract

Background: Autism Spectrum Disorder (ASD) is highly heterogeneous in etiology and manifestation. The neurobiological processes underlying ASD development are reflected in multiple features, from behavior and cognition to brain functioning. An integrated analysis of these features may optimize the identification of these processes.

Methods: We examined cognitive and adaptive functioning, and ASD symptoms between 8 and 36 months in 161 infants at familial high-risk for ASD (HR) and 71 low-risk controls (LR), and neural sensitivity to eye-gaze at 8 months in a subsample of 140 HR and 61 LR. We used linked independent component analysis to extract patterns of variation across domains and development, and selected the patterns significantly associated with clinical classification at 36 months.

Results: An early process at 8 months, indicating high levels of functioning and low levels of symptoms linked to higher attention to gaze shifts, was reduced in infants who developed ASD. A longitudinal process of increasing functioning and low levels of symptoms was reduced in infants who developed ASD, while another process suggesting a stagnation in cognitive functioning at 24 months was increased in infants who developed ASD.

Limitations: Although results show a clear significant trend relating to clinical classification, there was substantial overlap between groups.

Conclusions: We uncovered underlying processes acting together early in development and associated with clinical outcome. Results highlight the complexity of emerging ASD, which goes beyond the borders of clinical categories. Future work should integrate genetic data to investigate the specific genetic risks linked to these processes.

Introduction

Autism Spectrum Disorders (ASD) are behaviourally defined by difficulties in social-communication, restricted and repetitive patterns of behaviours and interests, and sensory anomalies¹. The intrinsic heterogeneity of ASD is evident at different levels of analysis and points to multiple underlying biological mechanisms leading to the disorder^{2,3}. Integration of information from multiple concurrent and longitudinal data might be crucial to decompose this variability⁴ and understand the complexity of ASD development. Data integration allows for a better understanding of the underlying biological mechanisms leading to different subgroups in phenotype by investigating their effects across multiple domains of functioning. This study aimed to uncover underlying processes early in development linked to later emergence of ASD. To do that, we looked for coherent patterns of variation across multiple developmental domains over time through an integrated analysis, in contrast to previous studies that have reported on categorical analyses that were only post-hoc associated across domains.

Prospective longitudinal studies of infants at familial high-risk for ASD (HR), based on having an older sibling with ASD, can inform on early manifestations of the disorder by investigating differences between infants who develop ASD and those who don't⁵. There is a general consensus in the field

that the defining behavioural features of ASD are not present in the first year of life but begin to emerge around 12 months and consolidate between 18 and 36 months^{6,7}. However, this pre-symptomatic period is characterised by sensorimotor⁸⁻¹⁰ and visual attention¹¹⁻¹⁴ atypicalities, and by alterations in brain structure¹⁵⁻¹⁷ and function¹⁸⁻²⁰ in infants with later ASD outcome. In particular, infants developing ASD demonstrate emerging atypicalities in social-communicative behaviour from the first year of life, with a declining interest in human faces²¹⁻²³ by 6 months of age. Event-related potentials (ERPs) provide a useful tool to examine the neural correlates of face recognition in infancy²⁴ through the characteristic P1, N290, and P400 components, known to be modulated by direction of eye-gaze as early as 4 months of age²⁵.

Although valuable to identify potential early risk markers for ASD, the traditional case-control comparison approach overlooks the heterogeneity of clinical outcome groups, which often overlap across symptoms²⁶. In fact, the idea of ASD as a discrete, separate entity can distort the investigation of the underlying mechanisms and early development of ASD.

Unsupervised data-driven methods are particularly advantageous when there is no a-priori knowledge on the actual sample subgroups²⁷ due to the absence of hypotheses for the inference of structure in unlabelled data. Here, we introduced a novel approach for prospective analysis of early development as

opposed to the more traditional retrospective investigation of early differences between categorical groups defined by ASD outcome. We separated underlying neurodevelopmental processes associated with clinical outcome based on the extraction of intrinsic patterns in multivariate unlabelled data through unsupervised learning methods. Our approach allows identifying different emerging patterns of development and investigating how they lead to specific outcomes by only looking at structure in the data. The identified patterns might then be the key to improve our understanding of individual heterogeneity and allow stratification into more homogeneous and predictable subgroups that might be a better target for early intervention. Compared to previous work on the same dataset¹⁰, this study shows a novel approach to prospective data. Our previous study used a more traditional analytic approach to examine differences in developmental trajectories between groups defined by current clinical categories, implicitly reinforcing existing clinical models. Here, we discover structure in data independently from clinical categories. Such approach potentially allows us to transform our understanding of the mechanisms underlying emerging ASD. Linked independent component analysis (ICA) can be used to simultaneously model and discover common features across multiple modalities²⁸⁻³⁰. Although mainly used in neuroimaging³¹⁻³³, this method can be directly applied to any type of

multimodal data acquired for a fixed group of participants. Applied to longitudinal multimodal data collected from large cohorts of infant siblings, it can help identify underlying biological processes with expression in different domains across development. In this study, we used linked ICA to uncover neurodevelopmental processes acting early in development by simultaneous factorization of developmental measures and electrophysiological measures of neural sensitivity to social and non-social stimuli at 8 months. The same approach was used to uncover underlying processes acting across development by simultaneous factorization of longitudinal developmental measures between 8 and 36 months. Then, we tested the post-hoc association of the identified processes to clinical outcome at 36 months. This provided novel insights into the neurodevelopmental processes acting together from early age and leading to different clinical outcomes depending on their presence at an individual level.

Methods and Materials

[Figure 1: Analysis flowchart]

We performed two separate analyses (Figure 1): a multimodal analysis to identify early neurodevelopmental processes, and a longitudinal analysis to identify processes acting across development.

Participants

Data were collected from infants recruited in one of two phases of the British Autism Study of Infant Siblings (BASIS, <http://www.basisnetwork.org>)^{18,34}, involving infants considered at high risk for ASD based on having an older biological sibling with ASD (HR siblings), and low-risk controls (LR). All procedures were in agreement with ethical approval granted by the London Central NREC (approval codes 06/MRE02/73, 08/H0718/76), and one or both parents gave informed consent to participate in the study. Experimenters were aware of infants' risk status, but assessments were blind to clinical outcome. At the time of enrolment, none of the infants had been diagnosed with any developmental condition.

The longitudinal sample included 232 infants (71 LR and 161 HR) followed on four visits at 8.1 ± 1.2 months (mean \pm standard deviation; hereafter 8 months), 14.5 ± 1.3 months (hereafter 14 months), 25.4 ± 3.1 months (hereafter 24 months) and 38.4 ± 2.3 (hereafter 36 months). To handle missing data, we performed imputation through expectation maximization on SPSS (<http://www.ibm.com/analytics/us/en/technology/spss>, see *Supplemental Material* for details). The multimodal analysis was run in a subsample of 201 infants (61 LR and 140 HR) selected because of having neural data available at 8 months (8.14 ± 1.22 months). Both samples were balanced in sex (see Table 1).

Measures

Developmental skills. Cognitive development was measured at each visit by the Mullen Scales of Early Learning (MSEL³⁵), a standardized developmental measure assessing cognitive functioning in 5 scales: gross motor (GM), visual reception (VR), fine motor (FM), receptive (RL) and expressive language skills (EL). T-scores (mean=50; standard deviation, SD=10) from the 5 scales at 8 months were included as input features in the multimodal analysis. Gross motor scores were excluded from the longitudinal analysis as not available at 36 months, leading to 4 input features from the MSEL.

Adaptive functioning. Adaptive behaviour was measured by the Vineland Adaptive Behavior Scales (VABS-II³⁶), a semi-structured parent-report questionnaire (at 8 and 14 months) or parent interview (at 24 and 36 months) assessing personal and social functioning in 4 different domains: Communication (Comm), Daily Living Skills (DL), Socialization (Soc) and Motor Abilities (Mot). Standard scores (mean=100; SD=15) from the 4 domains were included as input features in all analyses.

Early ASD symptoms. A 19-item version of the Autism Observation Scale for Infants (AOSI), a semi-structured observational assessment³⁷, was administered at 8 and 14 months to detect putative behavioural signs of ASD. The AOSI total score at 8 months was used as input feature in the multimodal analysis. To assess ASD symptomatology, the Autism Diagnostic Interview Revised (ADI-R³⁸) was administered at 36 months and

the Autism Diagnostic Observation Schedule (ADOS-2³⁹) was administered at 24 and 36 months. Total scores from the AOSI at 8 and 14 months and from the ADOS at 24 and 36 months were included in the longitudinal analysis.

Event-Related Potentials (ERPs). The task was the same as in Elsabbagh et al¹⁸. It was designed to assess responses to: static face [Fc]; visual noise stimuli [Ns]; static faces with direct gaze [FD]; static faces with averted gaze [FA]; gaze shifts toward the infant [SD]; gaze shifts away from the infant [SA]. Components P100, N290, and P400 averaged across occipito-temporal channels were quantified by amplitude and latency for a total of 36 ERP variables measured at 8 months and used as input features for the multimodal analysis (see *Supplemental Material* for details).

Clinical outcome evaluation at 36 months

The LR group was based on having an older full sibling with typical development. None of the LR infants met research criteria for ASD and none of them had a community clinical ASD diagnosis at 36 months (see *Supplemental Material* for details). Expert clinical researchers reviewed all available information on HR siblings at 24 months and 36 months and assigned clinical consensus best estimate diagnosis of ASD according to ICD-10⁴⁰ in Phase 1, and DSM-5 criteria¹ in Phase 2. The best estimate diagnoses for the two phases were reviewed for differences in categorization and considered

similar. HR siblings were subsequently grouped into siblings with ASD (HR-ASD); with atypical (non-ASD) development (HR-Atypical); and with typical development (HR-Typical) (see *Supplemental Material* for details).

Statistical Analysis

Linked ICA is a Bayesian extension of independent component analysis (ICA) for unsupervised learning of statistically independent modes of variation in data^{29,41}, allowing for the simultaneous analysis of multimodal data collected on the same participants²⁸. The identified components indicate processes considered independent based on how they affect different measures (i.e. across behavioural or neural data), but linked across modalities (i.e. behavioural versus brain data, Figure 1A) or time-points (Figure 1B). Each component explains variation within the individual participant and is represented by: (1) a vector of individual loadings, namely scalar values indicating how much that component explains developmental variation for the individual participant; (2) component weightings in different modalities; (3) a score map, indicating the relative value of scores compared to the estimated noise in individual variation. For the implementation, we used the code available on the FSL homepage (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLICA>). The number of independent components was estimated such that more than 90% of variance was explained.

In the multimodal analysis, we integrated measures of developmental level (10 total features from MSEL, VABS and AOSI) and ERP data at 8 months (36 total features; Figure 1A.1). The number of components was estimated to be 10. In the longitudinal analysis, we integrated developmental data (9 total features from MSEL, VABS and AOSI/ADOS) between 8 and 36 months. Different time-points were considered as different input modalities (Figure 1B.1) but were not considered as ordinal. The number of components was estimated to be 9.

We evaluated the association of the extracted components with clinical outcome through regression, with clinical outcome at 36 months as independent variable, individual component loadings as dependent variable, and sex as covariate (Figure 1A.3, 1B.3). We used Holm-Bonferroni correction to correct for multiple comparisons (Figure 1A.4, 1B.4). Differences in competence at different time-points, computed as average of MSEL and VABS scores, were tested via t-tests in robust ranges, and considered significant for $p < 0.05/6 = 0.008$ (tests=6).

Results

Data

Demographics are shown in Table 1, while clinical characteristics of the two samples can be found in the Supplemental Material (Table S3 and Table S4). Clinical

outcome groups did not differ in age at any visit, while sex was significantly different per clinical outcome ($\chi^2(3)=11.55$, $p=0.009$ in the multimodal analysis; $\chi^2(3)=9.66$, $p=0.022$ in the longitudinal analysis), with more males among the HR-ASD group.

[Table 1. Demographics]

Multimodal patterns of developmental and ERP data

[Figure 2. Independent component linked across modalities]

Among the 10 components across behavioural and brain data at 8 months, one was significantly associated to clinical outcome at 36 months (IC7: $\beta=-0.29$, $p<0.001$; Figure 2). This was a multimodal component (Figure 2C) showing a pattern in ERP variables (Figure 2A) characterized by longer P1 latency in response to gaze shifting away; higher P400 amplitude, lower P1 amplitudes and shorter N290 latency in responses to gaze shifts towards and away from the infant; and lower P1 amplitude in response to visual noise. The linked pattern in clinical measures at 8 months showed high levels of competence across all functional domains and low level of early ASD symptoms (Figure 2B). In particular, scores were higher in gross motor, visual reception, and receptive language MSEL scores, and communication and motor VABS scores. Individual loadings were negatively associated to clinical outcome ($\beta=-0.29$, Figure 2D), meaning that the identified process was

present more strongly in typical development. The effect of sex covariate was not significant after Holm-Bonferroni correction ($\beta=-0.37$ towards males, $p=0.007$).

Longitudinal patterns of developmental data

[Figure 3. Independent components linked across development]

Using longitudinal developmental measures, two components were significantly associated to clinical outcome at 36 months (IC1: $\beta=-0.60$, $p<0.001$; and IC3: $\beta=0.22$, $p<0.001$). IC1 (Figure 3, top row) was characterized by increasing competence across domains of cognitive and adaptive functioning between 8 and 36 months, reaching the peak in communication, daily living and social skills at 36 months, while the level of ASD symptoms was low over time (Figure 3A). Development of competence increased significantly between 8 and 14 months ($t(7)=-3.99$, $p=0.005$), and between 14 and 24 months ($t(7)=-8.25$, $p<0.001$), while the increase between 24 and 36 months was not significant ($t(7)=-2.73$, $p=0.029$) (Figure 3D). The identified process mostly explained variance from measures at 24 and 36 months (Figure 3B) and was negatively associated to clinical outcome ($\beta=-0.60$, Figure 3C), meaning that it was present more strongly in typical development. In fact, individual loadings on this component were higher in LR controls and HR-Typical than HR-Atypical and HR-ASD siblings (Figure 3C). The effect

of sex covariate was not significant ($\beta=-0.04$ towards males, $p=0.73$).

IC3 (Figure 3, bottom row) explained mostly variance on measures at 24 and 36 months (Figure 3F). It started with low levels of cognitive abilities at 8 months, followed by an increase in ASD symptom severity, visual receptive abilities and motor abilities (MSEL fine motor and VABS motor scores) by 24 months, and by a further increase in severity of ASD symptoms and a plateau in cognitive and adaptive functioning at 36 months (Figure 3E). In particular, average competence across cognitive and adaptive functioning decreased significantly between 24 and 36 months ($t(7)=5.07$, $p=0.004$; Figure 3H). There was a quadratic association between this pattern of scores and clinical outcome ($\beta_{\text{linear}}=0.19$, $\beta_{\text{quadratic}}=0.14$, Figure 3G), with a linear increase in individual loadings from HR-Typical to HR-ASD (Figure S1), but higher loadings in LR than HR-Typical siblings. Furthermore, there was a significant effect of sex covariate on clinical outcome, with more males than females among HR-Atypical and HR-ASD groups ($\beta=-0.40$, $p=0.002$).

Discussion

This study uncovers independent neurodevelopmental processes related to clinical outcome at 36 months. We presented a data integration approach to longitudinal developmental data and early brain measures to extract intrinsic patterns of

variation linked across domains. Contrary to retrospective group comparisons, such approach exploited the power of the prospective design by not having a priori assumptions on clinical categories. Then, we examined their relation to clinical outcome at 36 months.

By integrating clinical data and ERP responses to social stimuli at 8 months, we found a single neurodevelopmental process associated with clinical outcome at 36 months. At an individual level, this process explained more developmental variation in LR than HR-Atypical and HR-ASD groups, suggesting an association with typical development. The clinical pattern consisted in high levels of competence and low levels of symptoms. The neurophysiological correlates consist of a diffuse pattern of responses to gaze shifts, involving reduced and slower P1, increased P400 and faster N290 latency, but also reduced P1 to visual noise and slower P400 to direct gaze. This pattern suggests reduced attention capture but faster perceptual processing and deeper engagement to gaze shifts, and reduced attention capture by visual noise. Our previous work has already shown differences in P400 amplitude to dynamic gaze at 8 months between high-risk siblings with or without ASD outcome, and low-risk controls¹⁸. Here, we extended group comparison on single ERP measures to the identification of patterns from unlabelled data across integrated ERP measures linked to behavioural measures at the same age. We

found that higher neural engagement to a hard task like dynamic gaze shifts associates with high levels of visuomotor, but also communicative and social functioning at 8 months. This association might be explained by the complexity of gaze shift stimuli, which are likely more challenging for infants to process due to their dynamic nature involving rapid changes¹⁸. Furthermore, early sensitivity to dynamic gaze is fundamental to develop joint attention²⁵, which is thought to be crucial for cognitive, language and social development⁴². Greater attention to social stimuli might provide, in fact, increased opportunities for implicit social learning and the development of skills (e.g. learning words, interpreting facial expressions, predicting actions) underpinning typical development. However, the high overlap between groups in individual variation indicates that not all HR-ASD or atypical siblings were deviant on this pattern, which might rather define a subgroup. Interestingly, the process was mostly driven by ERP data (Figure 2.C), suggesting that ERP measures are more informative about clinical outcome than behavioural measures in infancy. This is likely because the ERPs can measure the early sensory and attentional alterations that are more commonly described as part of emerging ASD, while behavioural measures are probably too noisy and not specific to ASD in its prodromal phase^{6,7}.

By integrating longitudinal data from standardized clinical instruments, we aimed to capture pervasiveness of ASD symptoms in multiple functional domains. We found two processes significantly associated with clinical outcome. The first process indicated an increase in competence between 8 and 36 months accompanied by low levels of ASD symptoms. It occurs in a step-wise, sequential manner in which motor skills develop first, communication skills build on that and follow in development, followed in turn by social skills. This process was present more strongly in typical development, with decreasing scores going from LR controls to HR-ASD siblings. This is consistent with previous reports of developmental delay, poorer adaptive functioning and higher levels of ASD symptoms in HR non-ASD siblings⁴³. Furthermore, the HR-Atypical group was more instrument-defined than clinically based and included individuals with high variability in competence and/or ASD symptoms. Among them, some individuals might develop ASD later than 36 months of age, while others might show features of the Broad Autism Phenotype⁴³. Previous studies have already shown increasing trajectories of cognitive and adaptive functioning in LR and HR-Typical siblings^{8,10}. However, our approach to reveal this profile was novel. We only considered individual-level variation across measures over time and picked up this specific profile as explaining most of variance in data without any knowledge of clinical outcome. Thus, our results extend previous findings by showing that

this profile might actually represent an intrinsic developmental process underlying typical development. Previously observed differences between ASD and non-ASD siblings on single measures at different time-points might actually reflect a deviation from this underlying process. Furthermore, this process was highly correlated to the one obtained from the multimodal analysis at 8 months (see *Supplemental Material*). Thus, the neural pattern identified from ERP data at 8 months is likely to be associated to an increase in cognitive and adaptive functioning across development, indicating a pattern of increased developmental and neural functioning underpinning typical development.

The second pattern indicated a novel profile characterized by an increase in ASD symptoms over time and an early increase followed by a plateau in visual receptive and motor function between 24 and 36 months. This process was present more strongly in HR-ASD siblings and suggests a slower rate of gaining skills, or even stagnation over development. A more far reaching interpretation is that of regression, defined as the loss of acquired skills later in development, usually between 18 and 24 months, and the later emergence of impairments typical of ASD⁴⁴⁻⁴⁶. Recent studies have suggested that social-communication impairments were already present in infants before regression^{47,48}. Consistently, our pattern of late emerging ASD symptoms was linked to developmental

impairments already at 8 months, as shown by low Mullen scores in particular for receptive language. Furthermore, our findings support the recent hypothesis that regression might be a common process rather than an exception in ASD development^{45,46}. However, standardized scores make it difficult to distinguish regression from stagnation. It would be interesting to test whether this process could differentiate siblings who satisfied criteria for ASD already at 24 months from those who did only at 36 months. Of note, lower individual loadings in the HR-Typical than other clinical groups suggest that a reduced expression of the stagnation process, which entails strong cognitive skills in the first year of life, but slow visuomotor development and absence of overt ASD symptoms, promotes typical development. While previous neurophysiological studies investigated the superposition between liability to ASD and factors preventing ASD development⁴⁹, we identified a behavioural mechanism associated with reduced likelihood of developing ASD in infants with higher liability to ASD. Future research should integrate genetic and neurophysiological data, to improve our understanding of possible genetic or environmental factors associated with reduced likelihood of developing ASD in families with higher liability.

Taken together, our results highlight underlying developmental processes acting together in the first three years of life and

leading to different clinical outcome depending on their presence in the individual infant. We formally investigated intrinsic processes across developmental and brain data, in agreement with the general consensus on the necessity for data integration to improve our understanding of the underlying mechanisms for ASD. Our study adds to the literature by showing patterns of developmental variation linked across domains and across age that can help understand the unfolding of symptoms from the variety of early signs of ASD. The unsupervised approach is the strength of this study, which allowed us to pull apart different underlying processes expressing intrinsic variation in development independently from clinical categories. Although there is a priori evidence that the measures included would likely be associated with ASD outcome^{8,10,18}, our statistical approach had no a priori assumption the relationship between measures and clinical categories. This approach opens up various possibilities for the investigation of the biological processes acting early in development and preceding an ASD diagnosis. Future work could investigate the relation of the identified neurodevelopmental processes to different early risk factors through the integration of data from different modalities (e.g. MRI, fNIRS or eye-tracking). Similarly, incorporating genetic data could aid understanding of whether a specific process is linked more to common variation or to single gene mutations. This would provide insight into trajectories of gene expression and

mechanisms going from genetic risk, to neurobiological alterations and the cognitive and behavioural differences observed within ASD.

This study also has limitations. First, our longitudinal analysis included measures at 24 and 36 months used to inform clinical outcome evaluation at 36 months. However, the identification of underlying processes did not depend on clinical outcome as it was only used for post-hoc association. Nevertheless, process selection might have been biased, as shown by the fact that the identified longitudinal processes mainly explained variance at 24 and 36 months. Second, the majority in the investigated sample had a typical outcome, thus the processes identified might not capture the full variation in atypical development due to its underrepresentation in the sample. Third, we could not investigate the expression of neurodevelopmental processes over time as ERPs were only available at 8 months. For the same reason, we could not investigate the neurophysiological correlates of the stagnation process, which might inform on possible protective factors and should be the focus of future research. Fourth, ERP data were based on peak detection, which might be more prone to noise in infants ^{50,51}. Finally, although results show a clear significant trend relating to clinical outcome, there was substantial overlap between clinical groups. However, clinical categorization was not the ultimate goal of this

study, rather the investigation of underlying developmental pathways acting together in the individual infant, trans-diagnostically, and leading to a more typical or atypical outcome depending on their level of expression.

The processes identified inform on the underlying neurodevelopmental mechanisms associated with emerging ASD. Although our findings do not show underlying processes specific to ASD per se, they can help shaping our view on early ASD by showing that there is no sharp boundary between ASD and atypical development as the ASD phenotype goes beyond the limits of clinical categories set by the DSM-5.

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References

- 1 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th edn, (2013).
- 2 Lai, M. C., Lombardo, M. V. & Baron-Cohen, S. Autism. *Lancet* **383**, 896-910, doi:10.1016/S0140-6736(13)61539-1 (2014).
- 3 Jones, E. J., Gliga, T., Bedford, R., Charman, T. & Johnson, M. H. Developmental pathways to autism: a review of prospective studies of infants at risk. *Neurosci Biobehav Rev* **39**, 1-33, doi:10.1016/j.neubiorev.2013.12.001 (2014).
- 4 Jeste, S. S. & Geschwind, D. H. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nat Rev Neurol* **10**, 74-81, doi:10.1038/nrneurol.2013.278 (2014).
- 5 Szatmari, P. *et al.* Prospective Longitudinal Studies of Infant Siblings of Children With Autism: Lessons Learned and Future Directions. *J Am Acad Child Adolesc Psychiatry* **55**, 179-187, doi:10.1016/j.jaac.2015.12.014 (2016).
- 6 Piven, J., Elison, J. T. & Zylka, M. J. Toward a conceptual framework for early brain and behavior development in autism. *Mol Psychiatry* **22**, 1385-1394, doi:10.1038/mp.2017.131 (2017).
- 7 Shen, M. D. & Piven, J. Brain and behavior development in autism from birth through infancy. *Dialogues Clin Neurosci* **19**, 325-333 (2017).
- 8 Estes, A. *et al.* Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2~years of life. *J Neurodev Disord* **7**, 24 (2015).
- 9 Flanagan, J. E., Landa, R., Bhat, A. & Bauman, M. Head lag in infants at risk for autism: a preliminary study. *Am J Occup Ther* **66**, 577-585, doi:10.5014/ajot.2012.004192 (2012).
- 10 Bussu, G. *et al.* Prediction of Autism at 3 Years from Behavioural and Developmental Measures in High-Risk Infants: A Longitudinal Cross-Domain Classifier Analysis. *J Autism Dev Disord*, doi:10.1007/s10803-018-3509-x (2018).
- 11 Elsabbagh, M. *et al.* Disengagement of visual attention in infancy is associated with emerging autism in toddlerhood. *Biol Psychiatry* **74**, 189-194, doi:10.1016/j.biopsych.2012.11.030 (2013).
- 12 Shic, F., Macari, S. & Chawarska, K. Speech disturbs face scanning in 6-month-old infants who develop autism spectrum disorder. *Biol Psychiatry* **75**, 231-237, doi:10.1016/j.biopsych.2013.07.009 (2014).
- 13 Chawarska, K., Macari, S. & Shic, F. Decreased spontaneous attention to social scenes in 6-month-old infants later diagnosed with autism spectrum disorders. *Biol Psychiatry* **74**, 195-203 (2013).

- 14 Elison, J. T. *et al.* White matter microstructure and atypical visual orienting in 7-month-olds at risk for autism. *Am J Psychiatry* **170**, 899-908, doi:10.1176/appi.ajp.2012.12091150 (2013).
- 15 Hazlett, H. C. *et al.* Early brain development in infants at high risk for autism spectrum disorder. *Nature* **542**, 348-351, doi:10.1038/nature21369 (2017).
- 16 Shen, M. D. *et al.* Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. *Brain* **136**, 2825-2835, doi:10.1093/brain/awt166 (2013).
- 17 Shen, M. D. *et al.* Increased Extra-axial Cerebrospinal Fluid in High-Risk Infants Who Later Develop Autism. *Biol Psychiatry* **82**, 186-193, doi:10.1016/j.biopsych.2017.02.1095 (2017).
- 18 Elsabbagh, M. *et al.* Infant neural sensitivity to dynamic eye gaze is associated with later emerging autism. *Curr Biol* **22**, 338-342 (2012).
- 19 Jones, E. J. *et al.* Reduced engagement with social stimuli in 6-month-old infants with later autism spectrum disorder: a longitudinal prospective study of infants at high familial risk. *J Neurodev Disord* **8**, 7, doi:10.1186/s11689-016-9139-8 (2016).
- 20 Emerson, R. W. *et al.* Functional neuroimaging of high-risk 6-month-old infants predicts a diagnosis of autism at 24 months of age. *Sci Transl Med* **9**, doi:10.1126/scitranslmed.aag2882 (2017).
- 21 Maestro, S. *et al.* Attentional Skills During the First 6 Months of Age in Autism Spectrum Disorder. *Journal of the American Academy of Child & Adolescent Psychiatry* **41**, 1239-1245 (2002).
- 22 Osterling, J. & Dawson, G. Early recognition of children with autism: A study of first birthday home videotapes. *Journal of Autism and Developmental Disorders* **24**, 247-257, doi:10.1007/bf02172225 (1994).
- 23 Jones, W. & Klin, A. Attention to eyes is present but in decline in 2-6-month-old infants later diagnosed with autism. *Nature* **504**, 427-431 (2013).
- 24 de Haan, M. *Infant EEG and event-related potentials*. (Psychology Press, 2007).
- 25 Johnson, M. H. *et al.* The emergence of the social brain network: evidence from typical and atypical development. *Dev Psychopathol* **17**, 599-619, doi:10.1017/S0954579405050297 (2005).
- 26 Insel, T. *et al.* Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry* **167**, 748-751, doi:10.1176/appi.ajp.2010.09091379 (2010).
- 27 Loth, E. *et al.* The EU-AIMS Longitudinal European Autism Project (LEAP): design and methodologies to identify and validate stratification biomarkers for autism spectrum disorders. *Mol Autism* **8**, 24, doi:10.1186/s13229-017-0146-8 (2017).
- 28 Groves, A. R., Beckmann, C. F., Smith, S. M. & Woolrich, M. W. Linked independent component analysis for multimodal data fusion. *Neuroimage* **54**, 2198-2217, doi:10.1016/j.neuroimage.2010.09.073 (2011).

- 29 Smith, S. M. *et al.* Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* **23 Suppl 1**, S208-219, doi:10.1016/j.neuroimage.2004.07.051 (2004).
- 30 Llera, A., Wolfers, T., Mulders, P. & Beckmann, C. F. Inter-individual differences in human brain structure and morphology link to variation in demographics and behavior. *Elife* **8**, doi:10.7554/eLife.44443 (2019).
- 31 Douaud, G. *et al.* A common brain network links development, aging, and vulnerability to disease. *Proc Natl Acad Sci U S A* **111**, 17648-17653, doi:10.1073/pnas.1410378111 (2014).
- 32 Wolfers, T. *et al.* Refinement by integration: aggregated effects of multimodal imaging markers on adult ADHD. *J Psychiatry Neurosci* **42**, 386-394 (2017).
- 33 Franx, W. *et al.* Integrated analysis of gray and white matter alterations in attention-deficit/hyperactivity disorder. *Neuroimage Clin* **11**, 357-367, doi:10.1016/j.nicl.2016.03.005 (2016).
- 34 Hendry, A. *et al.* Developmental change in look durations predicts later effortful control in toddlers at familial risk for ASD. *J Neurodev Disord* **10**, 3, doi:10.1186/s11689-017-9219-4 (2018).
- 35 Mullen, E. M. Mullen Scale of Early Learning: AGS edition. *Circle Pines, MN: American Guidance Service Publishing* (1995).
- 36 Sparrow S, B. D. & D., C. Vineland adaptive behavior sscale: second edition. *Shoreview, MN: American Guidance Service* (2005).
- 37 Brian, J. *et al.* Clinical assessment of autism in high-risk 18-month-olds. *Autism* **12**, 433-456, doi:10.1177/1362361308094500 (2008).
- 38 Kim, S. H., Thurm, A., Shumway, S. & Lord, C. Multisite study of new autism diagnostic interview-revised (ADI-R) algorithms for toddlers and young preschoolers. *J Autism Dev Disord* **43**, 1527-1538, doi:10.1007/s10803-012-1696-4 (2013).
- 39 Lord, C. R., M.; DiLavore, P.; Risi, S.; Gotham, K.; Bishop, S. *Autism diagnostic observation schedule-2nd edition (ADOS-2)*. (Western Psychological Corporation, 2012).
- 40 World Health Organization. The ICD-10 classification of mental and behavioural disorders : diagnostic criteria for research. *Geneva : World Health Organization* (1993).
- 41 Jutten, C. & Herault, J. Blind Separation of Sources .1. An Adaptive Algorithm Based on Neuromimetic Architecture. *Signal Process* **24**, 1-10, doi:Doi 10.1016/0165-1684(91)90079-X (1991).
- 42 Mundy, P. & Newell, L. Attention, Joint Attention, and Social Cognition. *Curr Dir Psychol Sci* **16**, 269-274, doi:10.1111/j.1467-8721.2007.00518.x (2007).
- 43 Charman, T. *et al.* Non-ASD outcomes at 36 months in siblings at familial risk for autism spectrum disorder (ASD): A baby siblings research consortium (BSRC) study. *Autism Res* **10**, 169-178, doi:10.1002/aur.1669 (2017).

- 44 Barger, B. D., Campbell, J. M. & McDonough, J. D. Prevalence and onset of regression within autism spectrum disorders: a meta-analytic review. *J Autism Dev Disord* **43**, 817-828, doi:10.1007/s10803-012-1621-x (2013).
- 45 Boterberg, S., Charman, T., Marschik, P. B., Bolte, S. & Roeyers, H. Regression in autism spectrum disorder: A critical overview of retrospective findings and recommendations for future research. *Neurosci Biobehav Rev* **102**, 24-55, doi:10.1016/j.neubiorev.2019.03.013 (2019).
- 46 Ozonoff, S. & Iosif, A. M. Changing conceptualizations of regression: What prospective studies reveal about the onset of autism spectrum disorder. *Neurosci Biobehav Rev* **100**, 296-304, doi:10.1016/j.neubiorev.2019.03.012 (2019).
- 47 Ozonoff, S. *et al.* Diagnostic stability in young children at risk for autism spectrum disorder: a baby siblings research consortium study. *J Child Psychol Psychiatry* **56**, 988-998, doi:10.1111/jcpp.12421 (2015).
- 48 Bacon, E. C. *et al.* Rethinking the idea of late autism spectrum disorder onset. *Dev Psychopathol*, 1-17, doi:10.1017/S0954579417001067 (2017).
- 49 Belmonte, M. K., Gomot, M. & Baron-Cohen, S. Visual attention in autism families: 'unaffected' sibs share atypical frontal activation. *J Child Psychol Psychiatry* **51**, 259-276, doi:10.1111/j.1469-7610.2009.02153.x (2010).
- 50 Hoehl, S. & Wahl, S. Recording infant ERP data for cognitive research. *Dev Neuropsychol* **37**, 187-209, doi:10.1080/87565641.2011.627958 (2012).
- 51 Munsters, N. M., van Ravenswaaij, H., van den Boomen, C. & Kemner, C. Test-retest reliability of infant event related potentials evoked by faces. *Neuropsychologia* **126**, 20-26, doi:10.1016/j.neuropsychologia.2017.03.030 (2019).

Tables

Table 1. Demographics

	Overall	HR-ASD	HR-Atypical	HR-Typical	LR
Longitudinal analysis					
	n	n	n	n	n
	232	32	43	86	71
Sex*					
Male	118	24	23	38	33
Female	114	8	20	48	38
Age	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)
8 m	8.13 (1.22)	8.03 (1.12)	8.33 (1.06)	8.24 (1.21)	7.92 (1.35)
14 m	14.48 (1.27)	14.50 (1.32)	14.56 (1.20)	14.58 (1.29)	14.31 (1.26)
24 m	25.39 (3.06)	24.84 (1.63)	26.40 (4.25)	25.72 (2.31)	24.63 (3.30)
36 m	38.39 (2.32)	38.06 (1.90)	38.19 (2.05)	38.62 (2.29)	38.39 (2.69)
Multimodal analysis					
	n	n	n	n	n
	201	30	36	74	61
Sex**					
Male	99	23	18	30	28
Female	102	7	18	44	33

Age		mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)
	8 m	8.14 (1.22)	8.03 (1.05)	8.31 (1.09)	8.27 (1.16)	7.92 (1.41)

[#]*Note:* This table shows sex (count, *n*) and age by clinical outcome group. Data are reported separately for the sample included in the longitudinal analysis and the multimodal analysis.

[#]*Abbreviations:* ASD= autism spectrum disorder; HR = high-risk siblings; LR = low-risk controls.

* Significant difference of sex per clinical outcome: $\chi^2(3) = 9.66$, $p = 0.022$.

** Significant difference of sex per clinical outcome: $\chi^2(3) = 11.55$, $p = 0.009$.

Figure captions

Figure 1: Analysis flowchart. This figure illustrates the different steps of analysis for the extraction of underlying processes associated with clinical outcome at 36 months. Panel A illustrates the multimodal analysis, integrating clinical and ERP data both collected at 8 months, while panel B illustrates the longitudinal analysis, integrating behavioural data from standardized clinical instruments collected between 8 and 36 months.

Figure 2. Independent component linked across modalities. This figure illustrates the independent component linked across ERP and clinical data, both collected at 8 months, significantly associated to clinical outcome at 36 months (IC7). Panels A and B respectively show the associated sources of variation, namely score maps indicating the relative value of scores compared to the estimated noise, for ERP and clinical scores. Panel C presents the contribution of each measure to the component and D shows individual participant loadings to the component grouped by clinical outcome at 36 months.

Abbreviations: SA = averted gaze shift; SD = direct gaze shift; FA = static averted gaze; FD = static direct gaze; Fc = face with static gaze (average between direct and averted); Ns = visual noise; A = amplitude; L = latency; GM = gross motor scores (MSEL); VR = visual reception scores (MSEL); FM = fine

motor scores (MSEL); RL = receptive language scores (MSEL); EL = expressive language scores (MSEL); Cm = communication scores (VABS); DL = daily living scores (VABS); Sc = social scores (VABS); Mt = motor scores (VABS); Ao = AOSI total score; MSEL = Mullen Scales of Early Learning; VABS = Vineland Adaptive Behavior Scale; AOSI = Autism Observation Scales for Infants.

Figure 3. Independent components linked across development.

This figure shows results for the independent components obtained from the analysis of longitudinal clinical data: IC1 (top row) and IC3 (bottom row). Panels A and E show the associated sources of variation, namely score maps indicating the relative value of scores compared to the estimated noise, for clinical scores at different time-points respectively for the two independent processes identified. Similarly, panels B and F present the contribution of each time-point to the components, while panels C and G show individual participant loadings to the components grouped by clinical outcome at 36 months. Finally, panels D and H show the trajectories of average competence across all functional domains (VR, FM, RL, EL, Cm, DL, Sc, Mt) respectively for the two independent processes identified. The red line marks the median of scores as shown in panels A and E, and indicates: (D) a significant increase in average competence between 8 and 14 months (*, $p < .05$), reaching its peak at 24 months (***, $p < .001$); (H) a

significant decrease in average competence between 24 and 36 months (**, $p < 0.005$).

Abbreviations: VR = visual reception scores (MSEL); FM = fine motor scores (MSEL); RL = receptive language scores (MSEL); EL = expressive language scores (MSEL); Cm = communication scores (VABS); DL = daily living scores (VABS); Sc = social scores (VABS); Mt = motor scores (VABS); Ao = ASD symptoms as measured by the AOSI total score at 8 and 14 months, and ADOS total score at 24 and 36 months; MSEL = Mullen Scales of Early Learning; VABS = Vineland Adaptive Behavior Scale; AOSI = Autism Observation Scales for Infants; ADOS = Autism Diagnostic Observation Schedule