

# Uncovering neurodevelopmental paths to autism spectrum disorder through an integrated analysis of developmental measures and neural sensitivity to faces

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**Background:** Autism spectrum disorder (ASD) is highly heterogeneous in its etiology and manifestation. The neurobiological processes underlying ASD development are reflected in multiple features, from behaviour 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 and 71 low-risk controls; we also examined neural sensitivity to eye gaze at 8 months in a subsample of 140 high-risk and 61 low-risk infants. We used linked independent component analysis to extract patterns of variation across domains and development, and we 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, and another process suggesting a stagnation in cognitive functioning at 24 months was increased in infants who developed ASD. **Limitations:** Although the results showed a clear significant trend relating to clinical classification, we found substantial overlap between groups. **Conclusion:** We uncovered underlying processes that acted together early in development and were associated with clinical outcomes. Our results highlighted 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 disorder (ASD) is behaviourally defined by difficulties in social communication, restricted and repetitive patterns of behaviours and interests, and sensory anomalies.<sup>1</sup> The intrinsic heterogeneity of ASD is evident at different levels of analysis and points to multiple underlying biological mechanisms leading to the disorder.<sup>2,3</sup> Integration of information from multiple concurrent and longitudinal data might be crucial for breaking down this variability<sup>4</sup> and understanding the complexity of ASD development. Data integration allows for a better understanding of the underlying biological mechanisms that lead to different subgroups in phenotype by investigating their effects across multiple domains of functioning. This study aimed to uncover underlying processes early in development that are linked to the later emergence of ASD. To do that, we looked for coherent patterns of variation across multiple devel-

opmental domains over time through an integrated analysis, unlike previous studies that have reported on categorical analyses that were associated only post hoc across domains.

Prospective longitudinal studies of infants at familial high risk for ASD (based on having an older sibling with ASD), can provide information about early manifestations of the disorder by investigating differences between infants who develop ASD and those who do not.<sup>5</sup> 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.<sup>6,7</sup> However, this pre-symptomatic period is characterized by sensorimotor<sup>8–10</sup> and visual attention<sup>11–14</sup> atypicalities, and by alterations in brain structure<sup>15–17</sup> and function<sup>18–20</sup> in infants with who later develop ASD. In particular, infants who develop ASD demonstrate emerging atypicalities in social–communicative behaviour from the first year of life, with a declining interest in human faces<sup>21–23</sup>

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by 6 months of age. Event-related potentials (ERPs) provide a useful tool for examining the neural correlates of face recognition in infancy<sup>24</sup> through the characteristic P1, N290 and P400 components, known to be modulated by the direction of eye gaze as early as 4 months of age.<sup>25</sup>

Although the traditional case-control comparison approach is valuable for identifying potential early risk markers for ASD, it overlooks the heterogeneity of clinical outcome groups, which often overlap across symptoms.<sup>26</sup> In fact, the idea of ASD as a discrete, separate entity can distort investigation of the underlying mechanisms and early development of ASD. Unsupervised data-driven methods are particularly advantageous when there is no a priori knowledge of the actual sample subgroups<sup>27</sup> because of the absence of hypotheses for the inference of structure in unlabelled data. In this study, we introduced a novel approach for the prospective analysis of early development as opposed to the more traditional retrospective investigation of early differences between categorical groups defined by ASD outcomes. We separated underlying neurodevelopmental processes associated with clinical outcomes based on the extraction of intrinsic patterns in multivariate unlabelled data through unsupervised learning methods. Our approach allowed us to identify different emerging patterns of development and investigate how they led to specific outcomes by looking only at structure in the data. The identified patterns might then be the key to improving our understanding of individual heterogeneity and allow stratification into more homogeneous and predictable subgroups that could be better targets for early intervention. Compared with previous work on the same data set,<sup>10</sup> 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. In this study, we discovered structure in the data independent of clinical categories. Such an approach had the potential to transform our understanding of the mechanisms underlying emerging ASD.

Linked independent component analysis can be used to simultaneously model and discover common features across multiple modalities.<sup>28–30</sup> Although this method is used mainly in neuroimaging,<sup>31–33</sup> it 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, this approach can help identify underlying biological processes that are expressed in different domains across development. In this study, we used linked independent component analysis to uncover neurodevelopmental processes that are acting early in development by simultaneous factorization of developmental measures and electrophysiological measures of neural sensitivity to social and nonsocial stimuli at 8 months. We used the same approach 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 with clinical outcomes at 36 months. This provided novel insights into the neurodevelopmental processes that acted together from an early age and led to different clinical outcomes depending on their presence at an individual level.

## Methods

We performed 2 separate analyses (Fig. 1): a multimodal analysis to identify early neurodevelopmental processes, and a longitudinal analysis to identify processes that were acting across development.

### Participants

We collected data from infants recruited in 1 of 2 phases of the British Autism Study of Infant Siblings (BASIS; [www.basisnetwork.org](http://www.basisnetwork.org)),<sup>18,34</sup> involving infants considered to be at high risk for ASD because they had an older biological sibling with ASD (high-risk siblings), and low-risk controls. All procedures were in agreement with the ethical approval granted by the London Central NREC (approval codes 06/MRE02/73, 08/H0718/76), and 1 parent or both provided 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 low-risk and 161 high-risk) who were followed during 4 visits: at 8 months ( $8.1 \pm 1.2$  months, mean  $\pm$  standard deviation [SD]), 14 months ( $14.5 \pm 1.3$  months), 24 months ( $25.4 \pm 3.1$  months) and 36 months ( $38.4 \pm 2.3$ ). To handle missing data, we performed imputation through expectation maximization in SPSS ([www.ibm.com/analytics/us/en/technology/spss](http://www.ibm.com/analytics/us/en/technology/spss); for details, see Appendix 1, available at [jpn.ca/190148-a1](http://jpn.ca/190148-a1)). We ran the multimodal analysis in a subsample of 201 infants (61 low-risk and 140 high-risk), selected because they had neural data available at 8 months ( $8.14 \pm 1.22$  months). Both samples were balanced in terms of sex.

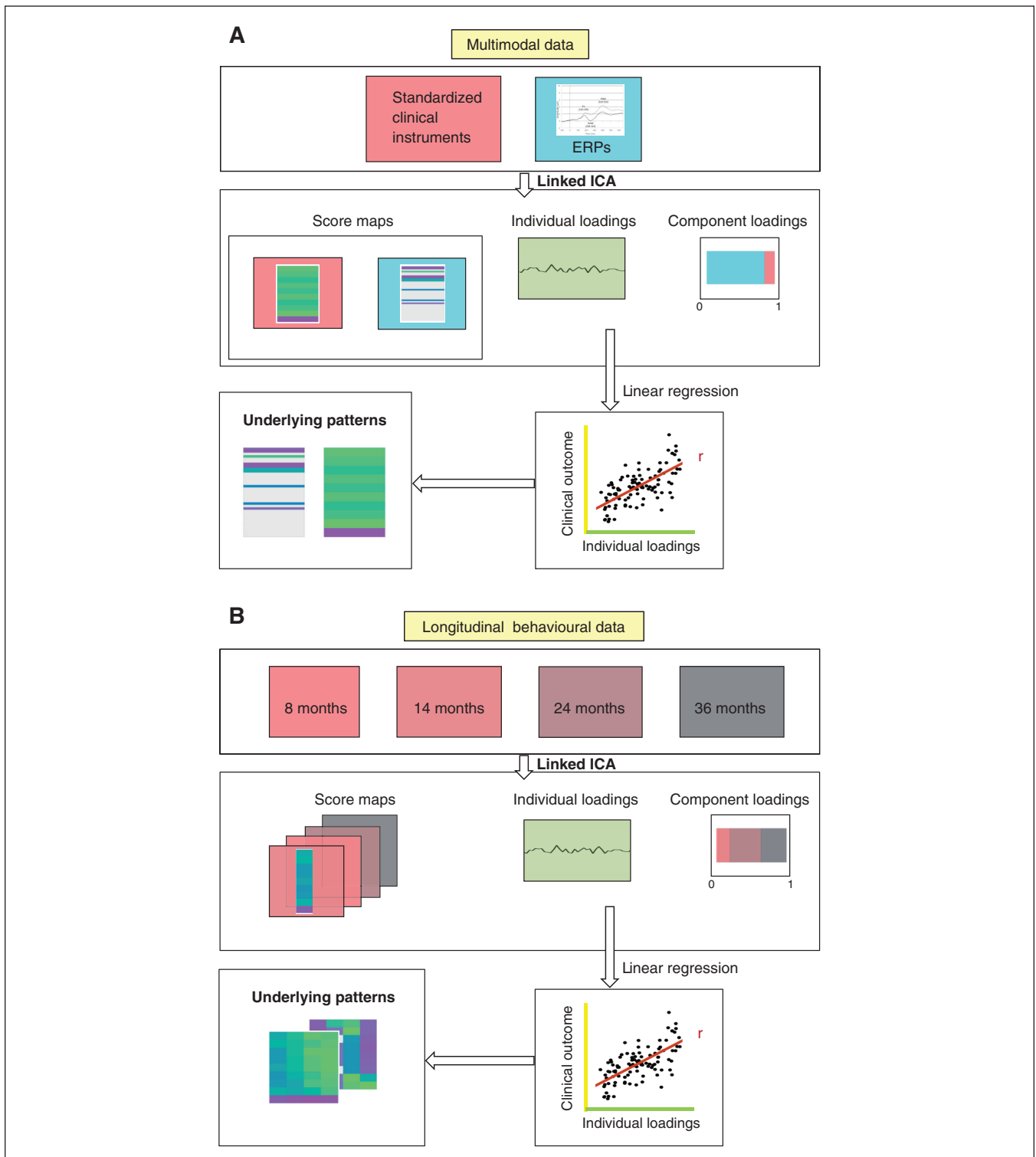
### Measures

#### Developmental skills

We measured cognitive development at each visit using the Mullen Scales of Early Learning (MSEL),<sup>35</sup> a standardized developmental measure that assesses cognitive functioning according to 5 scales: gross motor, visual reception, fine motor, receptive language and expressive language. We included T-scores ( $50 \pm 10$ , mean  $\pm$  SD) from the 5 scales at 8 months as input features in the multimodal analysis. We excluded gross motor scores from the longitudinal analysis because they were not available at 36 months, leading to 4 input features from the MSEL.

#### Adaptive functioning

We measured adaptive behaviour using the Vineland Adaptive Behaviour Scales (VABS-II),<sup>36</sup> a semistructured 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, daily living skills, socialization and motor abilities. We included standard scores ( $100 \pm 15$ , mean  $\pm$  SD) from the 4 domains as input features in all analyses.



**Fig. 1:** Different steps of analysis for the extraction of underlying processes associated with clinical outcome at 36 months. In particular, analyses started with different input features (measures  $\times$  participant) which were decomposed through linked independent component analysis (ICA) into the following (left to right): (1) score maps (measures  $\times$  component), indicating the relative value of scores compared to the estimated noise in individual variation; (2) individual participant loadings (components  $\times$  participant), indicating how much a component explained developmental variation for the individual participant; (3) component loadings in different modalities. Then, we tested the association of individual loadings with clinical outcomes through linear regression and corrected them for multiple comparisons to uncover underlying patterns (score maps  $\times$  modality) associated with the outcome of interest (here autism spectrum disorder). ERP = event-related potential.

### Early ASD symptoms

We administered a 19-item version of the Autism Observation Scale for Infants (AOSI), a semistructured observational assessment,<sup>37</sup> at 8 and 14 months to detect putative behavioural signs of ASD. We used the AOSI total score at 8 months as an input feature in the multimodal analysis.

To assess ASD symptomatology, we administered the Autism Diagnostic Interview–Revised<sup>38</sup> at 36 months and the Autism Diagnostic Observation Schedule (ADOS-2<sup>39</sup>) at 24 and 36 months. We included total scores from the AOSI at 8 and 14 months and from the ADOS-2 at 24 and 36 months in the longitudinal analysis.

### Event-related potentials

To evaluate event-related potentials (ERPs), we used the same task as used by Elsabbagh and colleagues.<sup>18</sup> This task was designed to assess responses to the following: static faces, visual noise stimuli, static faces with direct gaze, static faces with averted gaze, gaze shifts toward the infant, and gaze shifts away from the infant. We quantified components P100, N290 and P400 averaged across occipitotemporal channels by amplitude and latency for a total of 36 ERP variables measured at 8 months, and we used these as input features for the multimodal analysis (see Appendix 1 for details).

### Clinical outcome evaluation at 36 months

Those in the low-risk group had an older full sibling with typical development. None of the low-risk infants met research criteria for ASD, and none of them had a community clinical ASD diagnosis at 36 months (see Appendix 1 for details). Expert clinical researchers reviewed all available information on high-risk siblings at 24 and 36 months and assigned a clinical consensus, best-estimate diagnosis of ASD according to ICD-10<sup>40</sup> in phase 1, and DSM-5 criteria<sup>41</sup> in phase 2. We reviewed the best estimate diagnoses for the 2 phases for differences in categorization, and they were all considered similar. High-risk siblings were subsequently divided into groups of siblings with ASD (HR-ASD); with atypical (i.e., non-ASD) development (HR-atypical); and with typical development (HR-typical; see Appendix 1 for details).

### Statistical analysis

Linked independent component analysis is a Bayesian extension of independent component analysis for unsupervised learning of statistically independent modes of variation in data,<sup>29,41</sup> allowing for the simultaneous analysis of multimodal data collected on the same participants.<sup>28</sup> The identified components indicate processes considered to be 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; Fig. 1A) or time points (Fig. 1B). Each component explains variation within the individual participant and is represented by a vector of individual loadings, namely scalar values indicating how much that component explains developmental variation for the individual participant; component weightings in different modalities;

and a score map, indicating the relative value of scores compared with the estimated noise in individual variation. For implementation, we used the code available on the FSL homepage (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLICA>). We estimated the number of independent components 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-II and AOSI) and ERP data at 8 months (36 total features; Fig. 1A). We estimated the number of components to be 10. In the longitudinal analysis, we integrated developmental data (9 total features from MSEL, VABS-II and AOSI/ADOS-2) between 8 and 36 months. We considered different time points as different input modalities (Fig. 1B) but did not consider them to be ordinal. We estimated the number of components to be 9.

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

## Results

### Data

Demographic characteristics are shown in Table 1; clinical characteristics of the 2 samples can be found in Appendix 1, Tables S3 and S4. Clinical outcome groups did not differ in age at any visit, but sex was significantly different according to 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): there were more males in the HR-ASD group.

### Multimodal patterns of developmental and ERP data

Among the 10 components across behavioural and brain data at 8 months, 1 was significantly associated with clinical outcome at 36 months (IC7  $\beta = -0.29$ ,  $p < 0.001$ ; Fig. 2). This was a multimodal component (Fig. 2C) showing a pattern in ERP variables (Fig. 2A) characterized by longer P1 latency in response to gaze shifting away from the infant; higher P400 amplitude, lower P1 amplitudes and shorter N290 latency in response to gaze shifts toward 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 levels of early ASD symptoms (Fig. 2B). In particular, scores were higher for gross motor, visual reception and receptive language MSEL scales, and for communication and motor VABS-II scales. Individual loadings were negatively associated with clinical outcome ( $\beta = -0.29$ , Fig. 2D), meaning that the identified process was present more strongly in typical development. The effect of the sex covariate was not significant after Holm–Bonferroni correction ( $\beta = -0.37$  toward males,  $p = 0.007$ ).

**Table 1: Participant demographics\***

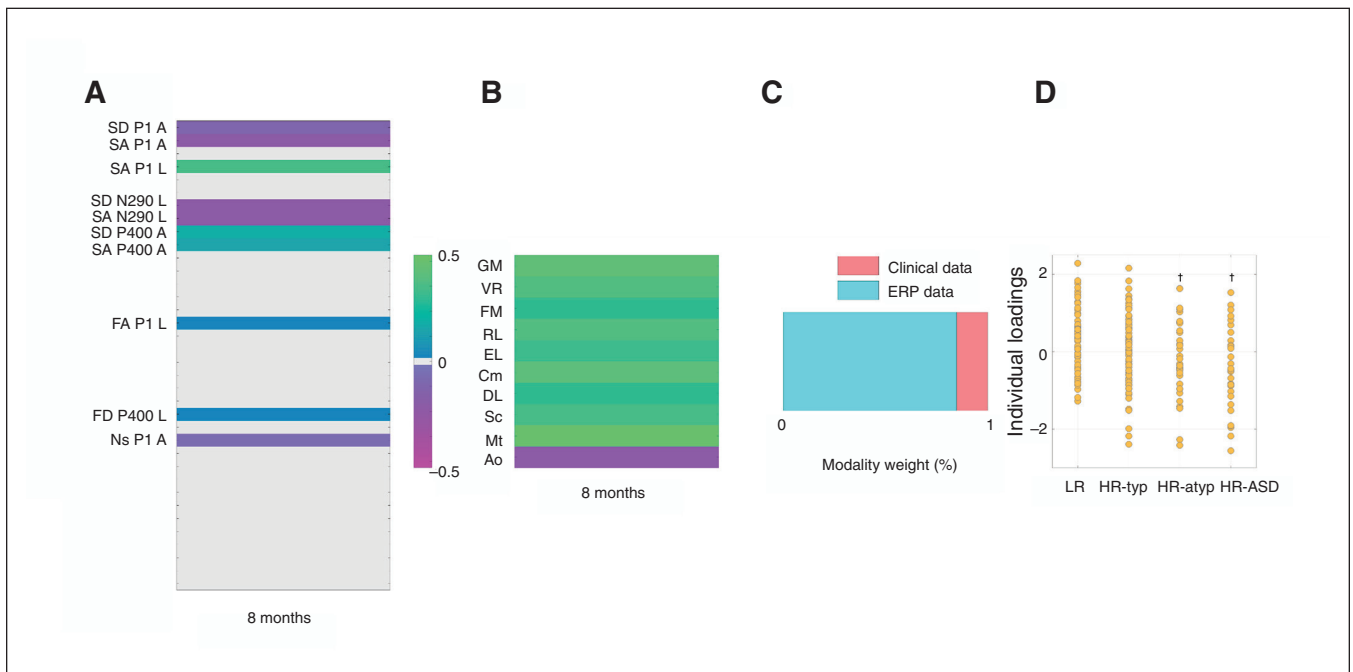
Characteristic	Overall	High-risk siblings, ASD	High-risk siblings, atypical	High-risk siblings, typical	Low-risk controls
<b>Longitudinal analysis</b>					
Participants, <i>n</i>	232	32	43	86	71
Sex, M/F†	118/114	24/8	23/20	38/48	33/38
Age, mean ± SD					
8 mo	8.13 ± 1.22	8.03 ± 1.12	8.33 ± 1.06	8.24 ± 1.21	7.92 ± 1.35
14 mo	14.48 ± 1.27	14.50 ± 1.32	14.56 ± 1.20	14.58 ± 1.29	14.31 ± 1.26
24 mo	25.39 ± 3.06	24.84 ± 1.63	26.40 ± 4.25	25.72 ± 2.31	24.63 ± 3.30
36 mo	38.39 ± 2.32	38.06 ± 1.90	38.19 ± 2.05	38.62 ± 2.29	38.39 ± 2.69
<b>Multimodal analysis</b>					
Participants, <i>n</i>	201	30	36	74	61
Sex, M/F‡	99/102	23/7	18/18	30/44	28/33
Age, mean ± SD					
8 mo	8.14 ± 1.22	8.03 ± 1.05	8.31 ± 1.09	8.27 ± 1.16	7.92 ± 1.41

ASD = autism spectrum disorder; SD = standard deviation.

\*This table shows sex (count, *n*) and age by clinical outcome group. Data are reported separately for the samples included in the longitudinal and multimodal analyses.

†Significant difference of sex per clinical outcome:  $\chi^2_1 = 9.66, p = 0.022$ .

‡Significant difference of sex per clinical outcome:  $\chi^2_3 = 11.55, p = 0.009$ .



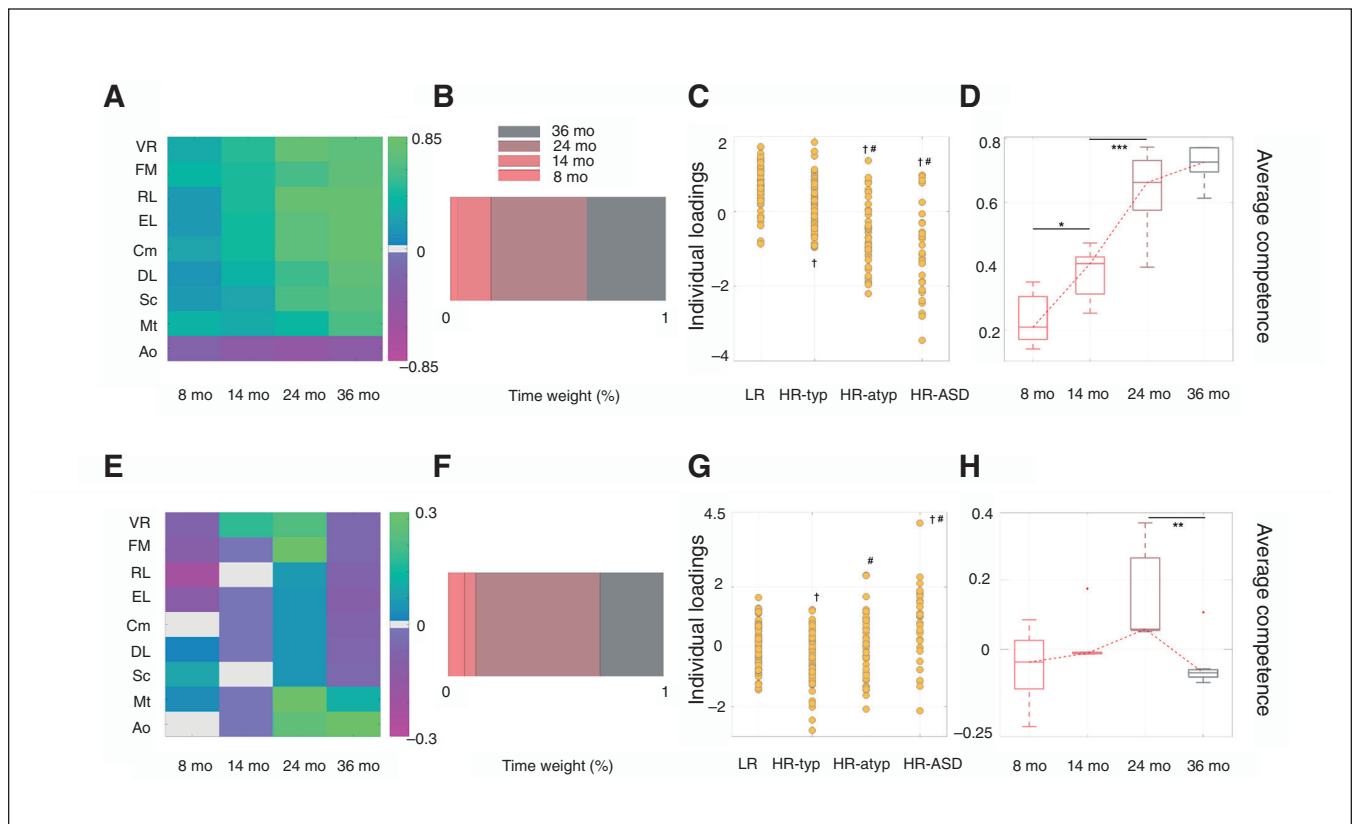
**Fig. 2:** Independent components linked across modalities. This figure illustrates the independent components linked across event-related potential (ERP) and clinical data, both collected at 8 months, significantly associated with clinical outcomes at 36 months (IC7). Panels A and B show the associated sources of variation, namely score maps indicating the relative value of scores compared with the estimated noise, for ERP and clinical scores, respectively. Panel C presents the contribution of each measure to the component. Panel D shows individual participant loadings to the component grouped by clinical outcome at 36 months. A = amplitude; Ao = AOSI total score; AOSI = Autism Observation Scales for Infants; ASD = autism spectrum disorder; atyp = atypical; Cm = communication scores (VABS); DL = daily living scores (VABS); EL = expressive language scores (MSEL); FA = static averted gaze; FD = static direct gaze; FM = finemotor scores (MSEL); GM = gross-motor scores (MSEL); HR = high-risk; IC = independent component; L = latency; LR = low-risk; MSEL = Mullen Scales of Early Learning; Mt = motor scores (VABS); Ns = visual noise; RL = receptive language scores (MSEL); SA = averted gaze shift; Sc = social scores (VABS); SD = direct gaze shift; typ = typical; VABS = Vineland Adaptive Behaviour Scale; VR = visual reception scores (MSEL).

Longitudinal patterns of developmental data

Using longitudinal developmental measures, we found that 2 components were significantly associated with clinical outcome at 36 months (IC1  $\beta = -0.60, p < 0.001$ ; and IC3  $\beta = 0.22, p < 0.001$ ). We found that IC1 (Fig. 3, top row) was characterized by increasing competence across domains of cognitive and adaptive functioning between 8 and 36 months, reaching a peak in communication, daily living and social skills at 36 months, and the level of ASD symptoms was low over time (Fig. 3A). Development of competence increased significantly between 8 and 14 months ( $t_7 = -3.99, p = 0.005$ ), and between 14 and 24 months ( $t_7 = -8.25, p < 0.001$ ); the increase between 24 and 36 months was not significant ( $t_7 = -2.73, p = 0.029$ ; Fig. 3D). The identified process mostly explained variance from measures at 24 and

36 months (Fig. 3B) and was negatively associated with clinical outcome ( $\beta = -0.60$ , Fig. 3C), meaning that it was present more strongly in typical development. In fact, individual loadings on this component were higher in low-risk controls and HR-typical than HR-atypical and HR-ASD siblings (Fig. 3C). The effect of the sex covariate was not significant ( $\beta = -0.04$  toward males,  $p = 0.73$ ).

We found that IC3 (Fig. 3, bottom row) primarily explained variance on measures at 24 and 36 months (Fig. 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-II motor scales) by 24 months, and by a further increase in severity of ASD symptoms and a plateau in cognitive and adaptive functioning at 36 months (Fig. 3E). In particular, average competence across cognitive and adaptive functioning



**Fig. 3:** Independent components linked across development. This figure shows results for the independent components (IC) 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 with the estimated noise, for clinical scores at different time points, respectively, for the 2 independent processes identified. Similarly, panels B and F present the contribution of each time point to the components, and 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) for the 2 independent processes identified. The red line marks the median of scores as shown in panels A and E, and indicates the following: (D) a significant increase in average competence between 8 and 14 months ( $*p < 0.05$ ), reaching its peak at 24 months ( $***p < 0.001$ ); (H) a significant decrease in average competence between 24 and 36 months ( $**p < 0.005$ ). ADOS = Autism Diagnostic Observation Schedule; Ao = ASD symptoms as measured by the AOSI total score at 8 and 14 months, and ADOS total score at 24 and 36 months; AOSI = Autism Observation Scales for Infants; ASD = autism spectrum disorder; atyp = atypical; Cm = communication scores (VABS); DL = daily living scores (VABS); EL = expressive language scores (MSEL); FM = fine-motor scores (MSEL); HR = high-risk; LR = low-risk; MSEL = Mullen Scales of Early Learning; Mt = motor scores (VABS); RL = receptive language scores (MSEL); Sc = social scores (VABS); typ = typical; VABS = Vineland Adaptive Behaviour Scale; VR = visual reception scores (MSEL).

decreased significantly between 24 and 36 months ( $t_7 = 5.07$ ,  $p = 0.004$ ; Fig. 3H). We found a quadratic association between this pattern of scores and clinical outcome ( $\beta_{\text{linear}} = 0.19$ ,  $\beta_{\text{quadratic}} = 0.14$ , Fig. 3G), with a linear increase in individual loadings from HR-typical to HR-ASD (Appendix 1, Fig. S1), but higher loadings in low-risk than in HR-typical siblings. Furthermore, we found a significant effect of sex on clinical outcome, with more males than females among the HR-atypical and HR-ASD groups ( $\beta = -0.40$ ,  $p = 0.002$ ).

## Discussion

This study uncovered 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. Unlike retrospective group comparisons, such an approach exploited the power of the prospective design by not placing a priori assumptions on clinical categories. Then, we examined their relationship with 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 low-risk controls than in the HR-atypical and HR-ASD groups, suggesting an association with typical development. The clinical pattern consisted of high levels of competence and low levels of symptoms. The neurophysiological correlates consisted 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 suggested reduced attention capture but faster perceptual processing and deeper engagement with 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 and low-risk controls.<sup>18</sup> Here, we extended the 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 difficult task like dynamic gaze shifts was associated with high levels of visuo-motor, communicative and social functioning at 8 months. This association might be explained by the complexity of the gaze shift stimuli, which are likely more challenging for infants to process because of their dynamic nature, involving rapid changes.<sup>18</sup> Furthermore, early sensitivity to dynamic gaze is fundamental for developing joint attention,<sup>25</sup> which is thought to be crucial for cognitive, language and social development.<sup>42</sup> In fact, greater attention to social stimuli might provide 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 indicated that not all HR-ASD or atypical siblings were deviant on this pattern; rather, it

might define a subgroup. Interestingly, the process was driven mostly by ERP data (Fig. 2C), suggesting that ERP measures are more informative about clinical outcomes than behavioural measures in infancy. This was likely because ERPs can measure the early sensory and attentional alterations that are more commonly described as part of emerging ASD; behavioural measures are probably too noisy and not specific to ASD in its prodromal phase.<sup>6,7</sup>

By integrating longitudinal data from standardized clinical instruments, we aimed to capture the pervasiveness of ASD symptoms in multiple functional domains. We found 2 processes that were significantly associated with clinical outcome. The first indicated an increase in competence between 8 and 36 months, accompanied by low levels of ASD symptoms. It occurred in a step-wise, sequential manner, in which motor skills developed first, communication skills built on that and followed in development, followed in turn by social skills. This process was present more strongly in typical development, with scores decreasing from low-risk controls to HR-ASD siblings. This finding was consistent with previous reports of developmental delay, poorer adaptive functioning and higher levels of ASD symptoms in HR non-ASD siblings.<sup>43</sup> 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 show features of the Broad Autism Phenotype.<sup>43</sup> Previous studies have already shown increasing trajectories of cognitive and adaptive functioning in low-risk and HR-typical siblings.<sup>8,10</sup> However, our approach to revealing this profile was novel. We considered only individual-level variation across measures over time and picked up this specific profile as an explanation for most of the variance in the data without any knowledge of clinical outcome. Thus, our results extend previous findings by showing that this profile might represent an intrinsic developmental process that underlies typical development. Previously observed differences between ASD and non-ASD siblings on single measures at different time points might reflect a deviation from this underlying process. Furthermore, this process was highly correlated with the one obtained from the multimodal analysis at 8 months (Appendix 1). Thus, the neural pattern identified from the ERP data at 8 months was likely to be associated with 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 suggested 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 ASD.<sup>44-46</sup> Recent studies have suggested that social-communication impairments are

already present in infants before regression.<sup>47,48</sup> Consistently, our pattern of late-emerging ASD symptoms was already linked to developmental impairments at 8 months, as shown by low MSEL scores, particularly for receptive language. Furthermore, our findings supported the recent hypothesis that regression might be a common process rather than an exception in the development of ASD.<sup>45,46</sup> However, standardized scores make it difficult to distinguish regression from stagnation. It would be interesting to test whether this process could differentiate siblings who already satisfied the criteria for ASD at 24 months from those who did so only at 36 months. Of note, lower individual loadings in the HR-typical group than in 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 visuo-motor development and an absence of overt ASD symptoms, promotes typical development. Although previous neurophysiological studies investigated the superposition between liability to ASD and factors preventing ASD development,<sup>49</sup> we identified a behavioural mechanism associated with a 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 the reduced likelihood of developing ASD in families with higher liability.

Taken together, our results highlight underlying developmental processes that act together in the first 3 years of life and lead to different clinical outcomes depending on their presence in the individual infant. We formally investigated intrinsic processes across developmental and brain data, in agreement with 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: it allowed us to pull apart different underlying processes that expressed intrinsic variation in development independent of from clinical categories. Although there is a priori evidence that the measures included would likely be associated with ASD,<sup>8,10,18</sup> our statistical approach had no a priori assumptions about the relationship between measures and clinical categories. This approach opens up possibilities for the investigation of the biological processes acting early in development and preceding an ASD diagnosis. Future work could investigate the relationship between identified neurodevelopmental processes and different early risk factors through the integration of data from different modalities (e.g., MRI, functional near-infrared spectroscopy 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 in ASD.

### Limitations

This study had several 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 outcomes; it was used only 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, so the processes identified might not capture the full variation in atypical development because of its under-representation in the sample. Third, we could not investigate the expression of neurodevelopmental processes over time, because ERPs were available only at 8 months. For the same reason, we could not investigate the neurophysiological correlates of the stagnation process, which might inform 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.<sup>50,51</sup> Finally, although our results showed a clear significant trend relating to clinical outcome, we found a substantial overlap between clinical groups. However, clinical categorization was not the ultimate goal of this study: rather, it was the investigation of underlying developmental pathways acting together in the individual infant, transdiagnostically, and leading to a more typical or atypical outcome depending on their level of expression.

### Conclusion

The processes we identified inform the underlying neurodevelopmental mechanisms associated with emerging ASD. Although our findings did not show underlying processes specific to ASD per se, they can help in shaping our view of 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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